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GENETIC CONTROL OF MUTATION INDUCTION IN SACCHAROMYCES CEREVISIAE

Jeffrey Fielding Lemontt
(Ph. D. Thesis)

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Genetic control of mutation induction in *Saccharomyces cerevisiae*

Jeffrey Fielding Lemontt

ABSTRACT

Twenty mutants of *Saccharomyces cerevisiae* were selected for reduced ultraviolet light (UV)-mutability as measured by locus reversion of the highly UV-revertible ochre-suppressible *arg4-17* allele. These mutants represent only three unlinked recessive loci denoted by *rev1*, *rev2* and *rev3*. The *rev2* locus is on chromosome XII at a distance of 4.0 centimorgans distal to *asp5*. Neither *rev1* nor *rev3* is centromere-linked. Strains carrying *rev1-1*, *rev2-1* and *rev3-1* are moderately UV-sensitive with Dose Reduction Factors (DRF) of 3.7, 4.3, and 8.5 in the respective haploids and 2 in the three homozygous *rev* diploids compared to wild-type; *rev1-1*, *rev2-1* and *rev3-1* also confer X-ray sensitivity (DRF's of 2.4, 1.5, and 1.8 at 10% survival in respective homozygous *rev* diploids) and ethylmethanesulfonate (EMS)-sensitivity (DRF's at 1% survival of 1.1, 2.7, and 2.7 in respective *rev* haploids). Mutation induction controlled by a small number of genes is thought

available on how these alterations are translated by the cell into mutations. Evidence reviewed by Bridges (7) indicates that most mutations are not induced solely because damage exists in the DNA, but rather arise as a result of metabolic repair processes acting on this damage. These processes are genetically controlled (7, 107). Studies in E. coli have revealed an intimate relationship between repair of lethal ultraviolet light (UV) damage in DNA and the production of UV-induced mutations (107). This has been possible because: 1) A major component of UV damage in nucleic acids was identified as cyclobutane pyrimidine dimers (101, 85). 2) The repair of such damage has been observed directly by biochemical assays independent of colony-forming ability (82, 73, 83). 3) Mutants exist that are known to be deficient in one or another of these repair processes (32, 33). It is possible that during a repair process errors are generated that may be the source of mutations. 4) UV-induced mutation has been studied in pairs of strains, one possessing and one lacking repair ability. The accuracy of various repair mechanisms has been inferred.

Photoreactivation (PR) (42, 43, 36, 46, 24), observed in a broad range of organisms, monomerizes pyrimidine dimers in DNA in the presence of both visible light and an intracellular enzyme (84). The phr mutants of E. coli (25) lack enzyme activity and are unable to effect repair. In these strains, it has been shown (109, 104, 44, 45) that at doses below 900 erg/mm^2, pyrimidine dimers are

responsible for more than 90% of the mutations induced by UV in the wild-type. Not all dimers are split by PR. Other non-dimer damage is also known to occur (91). Mutation studies in phr strains have also shown that PR itself does not generate the errors leading to the majority of UV-induced mutations. Witkin (107) has concluded that "if the splitting of a pyrimidine dimer can cause a mutation, an unsplit pyrimidine dimer is at least ten times more likely to cause one."

Since the first observation that UV-induced thymine dimers were released from the DNA of UV-irradiated E. coli (87, 6), impressive evidence (86) has been gathered to support an excision repair model. In such a model, short-single-stranded regions of DNA containing UV damage are recognized and removed enzymatically (41), followed by repair replication (73) and the eventual joining of strands (69, 21). Mutants of E. coli denoted as uvr or hcr are defective in the initial excision step (32). In studies using these mutants, it has been shown that at low UV doses mutations are induced with much higher frequencies in excision-defective strains compared to wild-type (105, 29). Witkin (107) has concluded that a mutation is 500 times more likely to arise from an unexcised pyrimidine dimer than from an excised dimer. Unexcised pyrimidine dimers are thought to cause virtually all of the UV-induced mutations in an excision-defective strain of E. coli. The excision repair mechanism has been estimated (107) to cause less than one mutation per million pyrimidine dimers excised. Thus,

it seems improbable that UV-induced mutations in E. coli are caused by errors introduced into the DNA during excision repair.

Rupp and Howard-Flanders (83) found that in excision-defective E. coli pyrimidine dimers are not permanent blocks to normal DNA replication. The newly synthesized daughter strands in the first DNA replication after UV exposure were found to contain single-strand breaks opposite each pyrimidine dimer in the parental strands. The molecular weight of the daughter strands increased during the hour following the first post-irradiation DNA replication. This phenomenon was interpreted as a new kind of repair process, post-replication repair. Strains carrying recA are not only UV-sensitive, X-ray sensitive and highly recombination deficient (32, 13), but are also unable to effect post-replication repair (93). The recA+ gene is thought to act in a post-replication repair mechanism dependent upon genetic recombination (35, 92) and operating independently of excision repair (35). Strains carrying recA produce no UV-induced mutations (58, 108). The recB, recC and exr loci in E. coli also cause UV and X-ray sensitivity and recombination deficiency, but to a much lesser degree than recA (32, 16). Like recA, exr blocks UV-induced mutation (106), while recB and recC reduce it significantly (108). Witkin (107) has interpreted such evidence in terms of a post-replication recombinational repair model: Most UV-induced mutations arise as errors introduced into the DNA during recombinational repair of

daughter strand breaks. The exr gene may interfere with the error-generating feature of this repair, while recA prevents any repair at all. The roles of recB+ and recC+ in UV-mutagenesis is unknown.

Very little is known from direct studies about the molecular basis of induced mutations in fungi. The main obstacle in this direction has been the inability to observe molecular changes in the DNA's of mutants blocked in mutation induction, since specific labeling of the DNA in many fungi including yeast is difficult to attain at present (22). An indirect approach to the problem of UV-mutagenesis in fungi has involved the possibility that such a process may be related to repair of lethal DNA damage, as in E. coli. Evidence that dark repair of lethal DNA damage occurs in yeast was first presented by Patrick et al. (70, 71). Attempts to recover mutants deficient in one or another DNA repair step have been mainly restricted to isolating radiation-sensitive strains (10, 14, 23, 30, 47, 50, 66, 78, 111, 77).

If UV-induced mutation in fungi occurs by a mechanism similar to that in bacteria, then some UV-sensitive mutants are expected to exhibit enhanced frequencies of UV mutation, while others should exhibit reduced frequencies compared to wild-type. Many UV-sensitive strains do exhibit enhanced UV mutation frequencies (2, 79, 111) like the excision-defective strains in E. coli. Others exhibit reduced frequencies (11, 68, 110) like the rec and exr strains

in E. coli. In addition, some of these mutants might be expected to affect recombination in varying degrees like the rec and exr strains in bacteria. In fungi, meiotic recombination in diploids is unaffected by homozygous mutations causing UV-sensitivity (10, 31, 47, 95). UV-sensitive genes have also been tested for their effect on induced mitotic recombination (64, 95, 111). At equal UV doses, induced mitotic recombination in diploids homozygous for these genes occurred at frequencies greater than in wild-type strains.

The mechanism of mutation induction in yeast is evidently complex. The molecular nature of the deficiencies associated with UV-sensitive mutants is unknown. A more straightforward analysis of mutation induction would be to isolate mutants that are defective in induced mutation. These might have interesting properties. If mutagenesis is dependent upon repair of lethal DNA damage, as in bacteria (107), then one would expect to find some that have properties of rec strains and some that have properties of exr strains. If mutagenesis can also proceed independently of the steps involved in the repair of DNA, one would also expect to find some mutants that have the full complement of repair functions. In In addition to those UV-sensitive genes in fungi that also reduce UV-mutation, new genes controlling mutation induction may be found. If mutation induction is found to depend upon DNA repair, then new mechanisms of repair might eventually be uncovered by this tactic.

In the present study, mutants of Saccharomyces cerevisiae were selected (49) directly for reduced ability to undergo UV-induced reversion with the aim of answering the following questions:

1) Are there any similarities between UV-induced mutation in yeast and in bacteria?

2) Does UV-mutagenesis in yeast depend upon the repair of lethal DNA damage?

3) How many genes are involved in the control of UV-mutagenesis in yeast?

4) Can such genes reduce all types of UV-induced mutation?

Mutants characterized by a reversionless phenotype (rev) were tested for a variety of phenotypic attributes. Complementation tests were performed among segregants. The specificity of the induced mutation phenotype of these genes was studied. The dependence of induced mutation on the repair of lethal DNA damage was investigated by testing mutants for the following: UV-sensitivity, X-ray sensitivity, EMS-sensitivity, and both UV-sensitivity and UV-induced reversion in multiple mutant strains. The role of recombination in UV-mutagenesis was also studied. Based on these results, the genetic control of mutation induction in yeast is discussed in relation to the model proposed for E. coli.

II.

MATERIALS AND METHODS

A. Yeast strains

The following three strains were obtained from Professor R. K. Mortimer: X1687-16C (a arg4-17 his5-2 trp5-48 lys1-1 ade2-1 leu1-12 met1-1), X1687-12B (α arg4-17 his5-2 trp5-48 lys1-1 ade2-1), and S288C (standard wild-type strain with α mating type). Reversionless mutants were induced in X1687-16C. Such mutants were denoted by a number suffix, e.g., X1687-16C-235. Strains S288C and X1687-12B were used in genetic testing of putative reversionless mutants. All strains denoted by a prefix "XY" were derived mainly from these three strains. Other strains obtained from Dr. Mortimer were used in mapping and other studies.

Genetic symbols used are those proposed at the Osaka Yeast Genetics Conference, Osaka, Japan (62). A three-letter abbreviation indicates phenotype or growth requirement. This is followed by a number to indicate the gene controlling that phenotype. This number is followed by a dash and another number which specifies the particular mutant allele of that gene, e.g., arg4-17.

B. Media

YEPD: 1% Yeast extract (Difco), 2% Bacto-peptone (Difco), 2% dextrose, and 2% agar (Difco). Liquid YEPD growth medium contained no agar.

Synthetic complete (C): 0.67% Yeast Nitrogen Base without amino acids (Difco), 2% agar (Difco), 2% dextrose, and the following supplements added before autoclaving: 20 mg/l L-arginine (AR), 20 mg/l L-histidine (HI), 20 mg/l L-tryptophan (TR), 20 mg/l adenine (AD), 20 mg/l L-methionine (ME), 20 mg/l uracil (UR), 30 mg/l L-leucine (LE), and 30 mg/l L-lysine (LY). Filter-sterilized L-threonine (THR) was added at 100 mg/l after autoclaving.

Omission media: Same as synthetic complete except for one or more metabolites omitted, e.g., C-AR, C-HI-TR.

Petite medium (PET): 3% glycerol, 0.025% dextrose, 1% yeast extract, 2% Bacto-peptone, and 2% agar. The petite phenotype was scored as the inability to utilize glycerol as a carbon source for growth.

Pre-sporulation medium (GNAP): 5% dextrose, 1% Yeast extract, 2.3% nutrient agar, 2% Bacto-peptone, and 0.5% agar.

Sporulation medium: 1% potassium acetate, 0.1% dextrose, 0.25% Yeast extract, and 2% agar.

Slant agar (YEPAD): 1% Yeast extract, 2% Bacto-peptone, 2% dextrose, 2% agar, 40 mg/l adenine. Many strains carried ade2. Occasionally, spontaneous mutation to a second adenine

requirement results in a growth rate greater than in the strain carrying ade2 alone (80). Adenine was added to slant agar to minimize secondary mutant accumulation.

Sorenson's buffer: KH_2PO_4 and Na_2HPO_4, 0.067 M, pH 8. The pH is determined by the relative proportions of the two salts.

C. Radiation sources

The X-ray source was a beryllium-window X-ray tube (Machlett OEG 60). The exposure dose rate to cells on agar was 250 roengten per second at 50 KVp and 25 milliamperes (unfiltered). The ultraviolet light source consisted of three 8-watt General Electric lamps (G8T5, 90% intensity at 2537 Å). The dose rate to cells on agar was 26.5 erg/mm^2/sec. On certain occasions, the UV dose rate was reduced to one-third this value using only one lamp.

D. Genetic analysis

1. Mating and sporulation. Diploid strains were routinely constructed by mating two haploid strains of different mating type. Small quantities of overnight, rapidly growing haploid cultures were mixed on a YEPD plate. Mating occurred usually after 3-4 hours. The fusing haploid cells could be observed microscopically. Using a glass needle micromanipulator, these zygotes were isolated from other cells and allowed to grow on YEPD agar. Upon incubation, a pure colony of diploid cells resulted, after which part of this colony

was cultured on slant agar, while the rest was transferred to GNAP for pre-sporulation. After overnight growth, the GNAP plate was replica-plated onto sporulation agar. Sporulation usually occurred after 2-4 days, as evidenced by the presence of asci. A small portion of this culture was suspended in 0.2 ml of a 1:40 solution of Glusulase (Endo Laboratories, Garden City, New York), an extract from the crop of Helix pomatia which contains an enzyme mixture that digests ascus walls but not spore capsules (37). After incubation at 30°C for 10-20 minutes, the suspension was centrifuged at high speed in a clinical bench top centrifuge for about 5 minutes. The supernatant was then pippetted off and the sediment resuspended in about 0.2 ml of distilled water. This suspension was used for genetic analysis as described below.

2. Tetrad analysis. This procedure involved the use of a glass needle and micromanipulation to separate the four spores from each ascus, followed by subsequent phenotypic analysis of all four ascosporal clones. Spores were separated in a rectangular array on a YEPD agar slab (37). Then the slab was placed onto a YEPD plate and incubated at 30°C for about 2 days to allow the spores to germinate and form spore clones. Photographs taken of the spore clones provide a permanent record of spore viability. Portions of these clones were then transferred to a YEPD master plate and incubated for 1-2 days. The master plate was then replica-plated to various omission agar or other media to score various phenotypes. The master plate was reincubated for 1-2 days to allow spore

cultures to grow up again and was subsequently refrigerated for storage and retrieval.

3. Random spore analysis. This procedure involved the use of high frequency sound to disrupt and separate spore tetrads into single spores. The Glusulase-treated suspension was added to 10 ml of distilled water and was sonicated (100 watt Ultrasonic Disintegrator, Measuring and Scientific, Ltd., London) for 3 minutes at 21 kilocycles per second. The suspension was then diluted and plated on YEPD to yield about 50-100 colonies per plate. After incubation, random spore clones were replica-plated to various media to score the various phenotypes.

4. Genetic mapping. Mortimer and Hawthorne (61) and Hawthorne and Mortimer (27) have described methods for the analysis of gene-gene and gene-centromere linkage. In a cross involving two genetic markers, e.g., AB x ab, three classes of asci can be distinguished after sporulation. They are Parental Ditype (PD) in which the four spore genotypes AB, AB, ab, ab reflect the configuration of two parental marker genes; NonParental Ditype (NPD) in which the distribution is Ab, Ab, aB, aB; and Tetratype (T) in which there are four different spore genotypes AB, Ab, aB, ab. Gene-gene linkage was detected by observing a PD : NPD ratio significantly greater than unity. Similarly, for unlinked gene pairs the ratios of PD : NPD : T asci are expected to be 1 : 1 : 4 (61). Gene-centromere linkage was detected by the observation of a second division

segregation frequency (SDS) frequency significantly less than 2/3. This frequency can be determined by observing the frequency of T asci when one of the two genes is known to be centromere-linked and its SDS frequency is also known. The relationship of these quantities can be expressed in the equation (72) $T = x + y - \frac{3}{2} xy$, where x equals the SDS frequency of the gene in question, y equals the SDS frequency of a known centromere-linked gene, and T equals the frequency of tetratype asci resulting from a cross in which both genes are segregating. When y is very close to zero, which is the case for the gene trp1 having an SDS frequency equal to 0.94% (61), then x is very nearly equal to T. These mapping procedures are usually carried out with complete tetrads. Random spore analysis may also be used in mapping.

5. Detection of rev genes in tetrads. The segregation of genes blocking UV-induced reversion could be detected at first only in strains also carrying highly revertible markers, such as arg4-17, his5-2 and lys1-1. If the diploid was homozygous for any or all of these markers, then all four spores in each ascus could be analyzed for their revertibility. The procedure involved replica-plating the YEPD master plate containing the tetrads onto two plates of the appropriate omission media, e.g., onto two C-AR plates. One plate received the Optimum Inducing Dose for UV-resistant haploids (see Materials and Methods, Part H), while the other served as a control for spontaneous reversion. After about 4 days of incubation

at 30°C, spore clone imprints in which reversion had taken place could be identified by revertant colonies growing on the irradiated replica. Observation of revertants indicated that these spores were not UV-sensitive and had full reversion ability. By contrast, strains carrying genes blocking reversion or conferring UV-sensitivity produced no induced revertant clones on the background replica.

E. Dose response measurements

1. Growth conditions. A small number of cells was streaked out onto YEPD to yield single colonies. The plate was incubated until single colony isolates appeared. Then as much of the entire single colony as possible was transferred into a test tube containing 10 ml of liquid YEPD. This inoculum was then shaken at 30° C for about 4-5 days. The remaining cells in the colony were transferred to another YEPD plate and allowed to grow up. After 1-2 days of incubation, this plate was replica-plated to omission media and other plates to test the phenotype of the culture. Thus, the phenotype of each strain cultured in this way was verified before the actual quantitative experiment was performed. Inoculation of the liquid YEPD with cells from a single clone assured that the inoculum contained a pure population of cells and not a heterogeneous population that may have arisen on the slant. This could occur, for example, by mutation to prototrophy conferring a selective growth

advantage, by suppression or secondary ade mutation.

The liquid YEPD culture was harvested in the following manner. The cells were centrifuged for ten minutes at medium speed (about 1500 rpm) in a centrifuge (International Centrifuge, Size 2, 3/4 H. P., International Equipment Co., Boston) capable of holding four 2 mm x 18mm culture tubes. The medium was decanted and the cells washed twice with 20 ml of distilled water. The cells were then resuspended in a small volume of distilled water, the actual volume depending on the required cell concentration. After 4-5 days of growth in YEPD, adding about 2 ml of distilled water in this last step usually resulted in titres between 6×10^8 and 10^9 cells/ml. These suspensions were then placed on ice and plated at various dilutions for the determination of survival and induced mutation as described below.

2. Survival. The survival curve for a strain after exposure to a lethal agent is operationally defined as the fraction of cells competent to form a colony after treatment as a function of the treatment dose. For UV and X-ray survival, the cells were first diluted and plated onto YEPD agar and then exposed to various radiation doses. When washed cell suspensions contained on the order of 10^9 cells/ml, controls were diluted usually by a factor of 10^6 and plated so that about 100 colonies would arise. For more extensive treatment, a larger number of cells was plated depending upon the degree of killing, so that a similar number of surviving clones would also arise

on the irradiated plates.

3. Induction of revertants. Cells carrying auxotrophic markers were tested for UV induction of revertants by plating large numbers (10^7 - 10^8) of cells onto the corresponding omission medium, exposing the plate to UV, incubating the plate for 4-7 days, and then counting the number of revertant clones. Control (zero dose) reversion frequencies were always assayed by plating similar numbers of cells, but without subsequent radiation treatment. Usually, duplicate plates at two different plating densities, about 10^7 and 10^8 cells/plate, were employed to achieve reliable counts over a wide range of reversion frequencies.

4. Complementation tests. Pairwise complementation tests for the reversion function were performed in the following manner: Meiotic segregants of reversionless mutants were chosen in both mating types. All strains also carried the arg4-17 allele. Every strain in the a mating type was crossed to every other in the α mating type using the usual square array technique. Instead of isolating single zygotes as described in Genetic Analysis, mass matings of parental strains were used. That is, small portions of fresh cultures of the parental strains were mixed together on YEPD and the plate incubated overnight at 30°C. Since diploids grow faster than haploids, such overnight growth served to enrich the diploid fraction in the total population. To further enrich the proportion of diploids, small portions of these overnight cultures were transferred to a new

YEPD grid and again incubated for 1-2 days. This procedure resulted in an almost entirely diploid population. Haploid cells which had not mated during this incubation period were strongly selected against on the basis of growth rate.

The YEPD plate containing the grid of enriched diploid cultures was then replica-plated onto two C-AR plates. One plate received 20 seconds of UV while the other (control) received none. In UV-resistant diploids this dose induces arginine revertants approximately 100-fold over the spontaneous frequency, while survival remains nearly 100%. Diploids in which complementation of the reversion function had occurred were identified by the presence of UV-induced arginine revertants after incubation for about 3 days. Absence of induced revertants was considered as evidence for noncomplementation of the reversion function.

F. Detection of locus and suppressor revertants

When ochre-suppressible alleles such as arg4-17 and lys1-1 are induced to revert to prototrophy, each prototroph may represent either an induced external suppressor or a wild-type gene, resulting from an alteration at the mutant allele site. Revertants of arg4-17, for example, were tested to determine whether or not the reversion was due to suppressor induction. Plates containing revertant clones on the selective medium, C-AR, were replica-plated to other omission media corresponding to the other ochre-suppressible alleles

present, e.g., C-LY if lys1-1 was also carried in the strain. If the revertant grew on at least one other omission plate, it was scored as a suppressor. Revertants not scored as suppressors by this method were considered as site revertants (53). Classification of the suppressors was not attempted. Since revertants were generally tested for suppressors on only one or two other omission plates, some suppressors in other classes may have gone undetected (60). Thus, the estimate of site revertants represents an upper limit.

G. Measurement of induced mutation.

The induced mutation frequency is expressed in units of mutations per survivor. That is, mutations are observed only in cells which survive the mutagen and grow to form a colony. The procedure employed in calculating induced reversion frequencies was as follows: Cells were plated and irradiated as described previously. The prototrophic colonies growing on the omission medium after incubation were considered to be revertants. In the case of induced reversion of ochre-suppressible alleles, plates with revertant clones were tested for the presence of suppressors as described previously. Let r equal the total number of revertants (locus and suppressor), N equal the number of cells plated, S equal the surviving fraction, and f equal the fraction of total revertants carrying suppressors. Suppressor reversion frequencies were calculated as rf/NS and locus reversion frequencies as r(1-f)/NS.

In the case of forward mutation induction, a different procedure was employed. Since auxotrophic mutants cannot be selected on the appropriate media, a large number of surviving clones on nonrestrictive medium (YEPD) must be examined separately for the detection of forward mutants. The total number of mutants observed divided by the total number of clones examined represents the forward mutation frequency. Since all cells not surviving the mutagen treatment are not examined for mutations, the per survivor basis of this frequency is implicit in the scoring procedure.

H. Determination of the Optimum Inducing Dose of UV

A given UV dose will increase the reversion frequency (per survivor) above the spontaneous level, but it will also inhibit colony forming ability in a fraction of the population. Thus, depending upon the relative rates of inactivation and reversion induction, the frequency of revertants (per cell plated) may decrease, remain constant or increase with UV dose. In the latter case, a maximum value is usually reached. The UV dose producing this maximum is called the Optimum Inducing Dose (OID). After the OID is determined for a particular strain, all derivatives of that strain obtained by mutagen treatment can be initially screened for induced reversion ability by observing the reversion response after exposure to the OID. Lack of reversion after the OID indicates that the given strain may be blocked in reversion induction. It may be only UV-sensitive, however,

and a quantitative test is required to distinguish between these alternatives.

Since arg4-17 exhibits a high UV-revertibility (76), reversion to prototrophy in arg4-17 strains provided a convenient system for detecting mutants unable to undergo reversion. For maximal discrimination of putative reversionless mutants from wild-type, the OID of strain X1687-16C was measured for arginine reversion. Approximately 2 x 10^6 cells were plated onto each of a number of C-AR plates. The plates were then exposed to varying doses of UV and incubated. The number of arginine revertants per plate was determined. The data are shown in Figure 1. On the basis of this experiment, 345 erg/mm^2 was chosen as the OID.

I. Induction and selection of reversionless mutants

Cells of the strain X1687-16C were treated with 3% ethylmethanesulfonate (EMS) for one hour according to a similar method employed by Lindegren et al. (51). Cells from an overnight culture on YEPD agar were suspended in 9.2 ml of 0.067 M Sorenson's buffer (pH 8) at approximately 10^7 cells/ml. Then 0.5 ml of 40% dextrose solution and 0.3 ml of EMS were added to the suspension and shaken gently at 30°C for one hour, after which 0.2 ml was removed and put into 9.8 ml of 6% sodium thiosulfate solution to inactivate the EMS. After at least 10 minutes in sodium thiosulfate, the cells were diluted further and plated onto YEPD to yield 50-100 clones per plate.

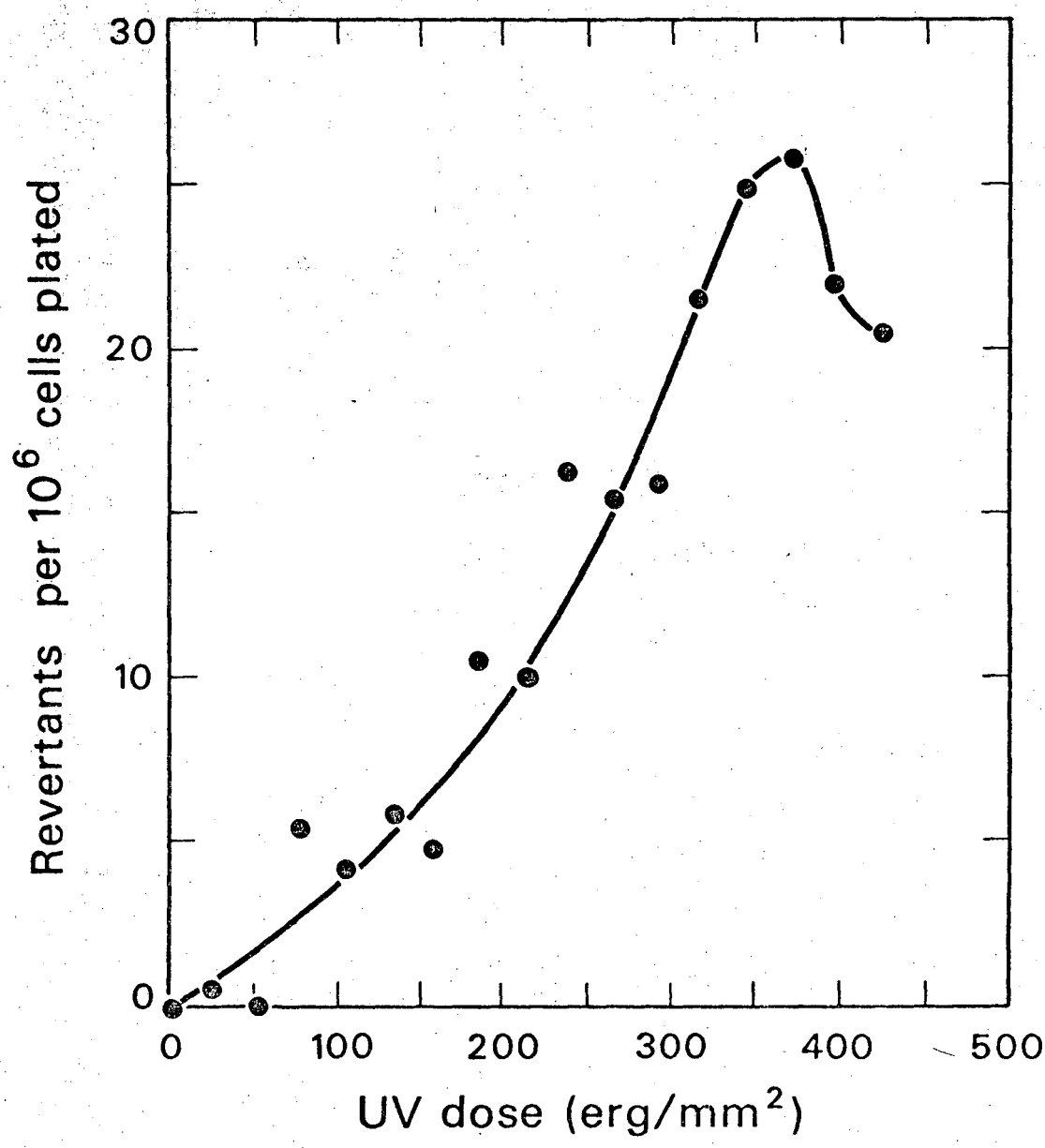

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Figure 1.--Reversion frequency of arg4-17 as a function of UV dose in X1687-16C.

Each YEPD plate was replica-plated onto two C-AR plates, after about 4-5 days of incubation. One of the replicas received the OID, while the other served as a control for spontaneous reversion. After about 4 days of incubation in the dark at 30°C, between 10 and 20 revertant clones per colony replica were observed on the UV-treated plate, but none or very few were formed on the control plate. Most colony replicas exhibited these UV-induced revertants, but some failed to show any or only a very few. The clones on the YEPD master plate corresponding to these nonreverters were identified and portions transferred to a new YEPD master plate for further testing.

J. Exclusion tests

Initially, a rev mutation could only be detected by observing its effect on the reversion of another mutant allele, e.g., arg4-17. However, several other alterations could simulate the phenotype expected of a rev mutation. Such false rev mutations could include mutation at another arginine gene, at a gene controlling the synthesis of a product not in the synthetic medium, at another allele in arg4, and a mutation to UV-sensitivity. The following tests were carried out on putative rev mutants to evaluate these possibilities:

1. Preliminary screening. Reversionless mutants were re-tested twice for the reversionless phenotype. In the first test, the YEPD master was also replica-plated onto synthetic complete agar

in addition to C-AR. Those mutants unable to grow on complete were excluded from further study. Such mutants would not be expected to produce revertants of arg4-17 on C-AR. In this case, lack of reversion was attributed to this new secondary growth requirement. Also excluded were mutants exhibiting reversion on retesting. Presumably, these represent phenotypic overlaps due to sampling error. In the second test, the remaining mutants were retested for the capacity to produce UV-revertants to arginine independence. Those isolates producing none or very few revertants were retained for further testing.

2. Suppressibility test. As a second screening procedure, suppressor mutations were selected on C-HI-TR medium in reversionless strains. These suppressors were then tested for growth on C-AR. The procedure involved plating a large number of cells (greater than 10^8 cells) onto C-HI-TR agar and incubating until prototrophic colonies arose. These colonies were considered to carry ochre-suppressors, arising spontaneously, since two ochre-suppressible markers, his5-2 and trp5-48, had apparently reverted simultaneoulsy. Plates containing these suppressor-bearing colonies were replica-plated onto C-AR. Lack of growth on C-AR indicated the probable existence of another defective arginine locus or another defective arg4 allele. Such reversionless strains were excluded.

3. Cross to wild-type. To conclusively determine the arginine genotype of reversionless strains, crosses were made to the wild-type S288C. After tetrad analysis a ratio of two spores growing to two spores not growing on C-AR in all asci indicated that the arginine requirement was confined to the arg4 locus. The reversionless phenotype was scored in tetrads as described previously. Random segregation of the reversionless phenotype relative to the arginine requirement indicated that UV-reversion was blocked by a gene that was not linked to the arg4 locus.

4. Single gene segregation. The remaining strains were crossed to strain X1687-12B. In these diploids arg4-17 was homozygous and UV-induced reversion of this allele was examined in every segregant. Observation of a 2 : 2 segregation pattern of reversion : no reversion in all asci after UV exposure to the OID provided evidence that a single nuclear gene difference was responsible for lack of reversion. The SDS frequency was also estimated for the reversionless gene relative to a heterozygous, centromere-linked marker, leu1-12. A value significantly less than 2/3 indicated that the locus preventing reversion was linked to a centromere (27).

5. Quantitative reversion induction. The entire isolation procedure was considered complete only when each tentative reversionless mutant was shown to exhibit a reversion frequency lower than wild-type, on a per survivor basis, for every UV dose. A UV-sensitive strain may produce many revertants, but a large fraction would be

inviable after receiving the OID for resistant strains. The OID for UV-sensitive strains would be considerably smaller, since the OID is a function of UV-sensitivity. Resnick (76) isolated a number of UV-sensitive mutants in yeast and found that reversion induction curves for a number of known alleles rose faster than control in these strains. Tentative reversionless strains were grown on YEPD and tested for UV survival and induced reversion of arg4-17, according to the procedures described in Dose-response measurements. Strains showing a locus reversion frequency equal to or greater than control (X1687-16C) at every dose were excluded. This includes those for which the apparent reversionless phenotype is due only to greater UV-sensitivity. Those exhibiting lower locus reversion than control were considered to be defective in UV-induced mutation.

III.

RESULTS

A. Isolation and allelism of reversionless mutants

Reversionless mutants were isolated in two separate experiments. In the first experiment, approximately 28,000 clones surviving the EMS treatment were tested for UV-induced reversion. The EMS survival was about 50%. There were 262 clones that exhibited the reversionless phenotype. After all false mutants were excluded, five remained. As mentioned previously, the overall selection procedure included genetic testing of reversionless mutants to determine that the reversionless character in every mutant was due to the presence of a single locus. Such loci exhibited the expected 2 : 2 segregation pattern when heterozygous in diploids. The five mutants isolated were given identification numbers referring to the order in which they were selected: X1687-16C-235, -63, -184, -255, and -10. The notation -63, for example, is an abbreviated form of X1687-16C-63.

To determine whether any of the reversionless strains might be allelic, complementation tests were performed. The results for the first group are shown in Figure 2. Mutants -184, -255, and -10 do not

a \ α	XY6-2C (−235)	XY2-1B (−63)	XY19-6D (−184)	XY82-7B (−255)	XY84-3C (−10)
XY6-2B (−235)	—	+	+	+	+
XY2-2C (−63)	+	—	+	+	+
XY19-4C (−184)	+	+	—	—	—
XY82-1C (−255)	+	+	—	—	—
XY84-5D (−10)	+	+	—	—	—

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Figure 2.--Complementation tests for reversionless strains. The numbers in parentheses identify the original mutants isolated. Strains used here are emeiotic segregants of these mutants. Minus sign indicates lack of arg+ revertants in diploid cultures after UV exposure of 20 seconds (530 erg/mm^2). Plus sign indicates presence of UV-induced revertants.

complement one another but do complement -235 and -63. Each of these two complement all of the other four. Thus, the five mutants fall into three complementation groups. Mutants -235 and -63 each represent the first two groups, while mutants -184, -255 and -10 form the third group.

To determine whether these three complementation groups correspond to three separate genes, diploids involving all pairwise combinations of the five mutants were sporulated. In Table 1a, random spore analysis of homoallelic diploids indicates that the frequencies of spores exhibiting wild-type reversion is very small. Diploids exhibiting complementation for the reversion function produce reverting spores at frequencies significantly greater than zero. Some of these are significantly different from the expected value of 0.25. Since the spore clones were not selected to exclude diploids, contamination of the random spore sample with unsporulated diploids probably occurred. If this contamination was appreciable, the frequency of reverting clones would be higher than 0.25, since these diploids undergo complementation and therefore do exhibit reversion. Also, variations in the PD : NPD ratio would increase or decrease the frequency of reverting clones from the expected 0.25 as discussed below.

Let a and b be reversionless genes in crosses a x b. After sporulation, PD, NPD and T asci would be produced with the following phenotypes:

Table 1a.--Random spore analysis of diploids from the complementation tests

Diploid	Total No. of Spores	No. Rev+		No. Rev+/Total		X^{2*}	$P(X^2)$
		Observed	Expected*	Observed	Expected*		
235x235	209	0	0	0	0		
63x 63	316	2	0	0.0064	0		
184x184	216	4	0	0.019	0		
255x255	336	6	0	0.018	0		
10x 10	809	2	0	0.0025	0		
235x 63	648	98	162	0.15	0.25	33.7	<0.01
235x184	139	27	35	0.19	0.25	2.4	>0.10
235x255	151	52	38	0.34	0.25	6.9	<0.01
235x 10	786	100	197	0.13	0.25	63.8	$\ll 0.01$
63x184	268	54	67	0.20	0.25	3.4	>0.05
63x255	392	37	98	0.09	0.25	50.7	$\ll 0.01$

*See text.

Table 1b.--Tetrad analysis of diploids from the complementation tests

Diploid	No. of asci	PD	NPD	T	%T
63x10	8	3	0	5	0.63
184x255	11	11	0	0	0
184x10	10	10	0	0	0
255x10	6	6	0	0	0

PD	UV-reversion	NPD	UV-reversion	T	UV-reversion
a+	-	ab	-	ab	-
a+	-	ab	-	a+	-
+b	-	++	+	+b	-
+b	-	++	+	++	+

The number of spore clones exhibiting UV-reversion in the PD, NPD, and T asci are 0, 2 and 1, respectively. Two unlinked genes are expected to yield ratios of PD : NPD : T equal to 1 : 1 : 4. Thus, 1/4 of all random spores revert. An increase in the PD : NPD ratio because of linkage, however, can also decrease the frequency of reverting spores from the expected 0.25. Thus, -235, -63 and -184 were all considered to carry single, unlinked reversionless loci. In Table 1b diploids not exhibiting complementation were found to produce only PD asci when analyzed by tetrad analysis and were therefore considered to be allelic. On the basis of these results, the genes responsible for the reversionless phenotype in mutants -235, -63, -184, -255 and -10 were denoted as rev1-1, rev2-1, rev3-1, rev3-2 and rev3-3, respectively.

A second group of mutants was then isolated. In the second experiment, a slightly different procedure was employed to facilitate the isolation of new mutant loci. About 9000 clones were screened for reversion as described previously. The EMS-survival was about 30%. There were 330 clones that exhibited the reversionless phenotype. These mutants were subjected to preliminary screening tests (see Materials and Methods: preliminary screening

and suppressibility tests) to exclude most false mutants. There were 300 mutants excluded from further consideration by these tests. The remaining 30 reversionless mutants were tested for ability to complement three strains each carrying one of the following alleles: rev1-1, rev2-1 and rev3-1. Of these 30, two did not complement rev1-1, two did not complement rev2-1 and 11 did not complement rev3-1. Such noncomplementation was considered to be due to allelism of the strains involved. The remaining 15 complemented all three testers and were subjected to further testing.

Each of these 15 mutants was crossed to the wild-type strain S288C. After sporulation, the spore clones were analyzed by tetrad analysis. Three of these mutants failed to exhibit a 2 : 2 segregation pattern for the arginine requirement. This indicated that the arg4-17 allele was not the only arginine locus controlling arginine biosynthesis in the mutant. Since this probably accounted for the reversionless phenotype, these three mutants were excluded from further study. The remaining 12 reversionless mutants, however, did exhibit a 2 : 2 segregation pattern among the spore clones in each tetrad. The reversionless character was not linked to the arginine requirement, i.e., arg4, in these tetrads.

Meiotic segregants of these 12 mutants were isolated in both mating-types and pairwise complementation tests for the UV-reversion function were carried out to determine if any of these 12 mutants were allelic. There were only two mutants that did not

complement, i.e., failed to produce UV-revertants of arg4-17 in the diploid. Thus, of 12 putative reversionless mutants, there were 11 complementation groups, each group being different from the three groups separately defined by rev1, rev2 and rev3.

Eight of these 12 mutants (including the two allelic mutants) were later found to be UV-sensitive and exhibited Optimum Inducing Doses much lower than the wild-type strain. These were excluded since they had reversion ability. Since they were also UV-sensitive, however, UV-induced revertants were inviable after the Optimum Inducing Dose for the wild-type strain. The remaining four mutants were only moderately UV-sensitive but all exhibited a quantitative UV-reversion response (per survivor) equal to or greater than the strain wild-type for reversion. These remaining four mutants were also excluded.

In summary, 20 rev mutants were isolated. Three were found to carry a gene denoted as rev1, three carried a gene denoted as rev2 and 14 carried a gene denoted as rev3. Table 2 shows a list of the mutants isolated and their corresponding allele designations.

Of these 20 mutants, five were identified on the first trial from a total of about 28,000 clones when the EMS survival was about 50%. Fifteen were identified on the second trial from a total of about 9000 clones when the EMS survival was about 30%. The difference in induction frequencies was probably due to the difference in survival levels. A lower survival level after EMS treatment would yield a

Table 2.--Allele designations of reversionless mutants

Mutant Number	Allele Designation
X1687-16C-235	rev1-1
-265	rev1-2
-575	rev1-3
X1687-16C- 63	rev2-1
-311	rev2-2
-538	rev2-3
X1687-16C-184	rev3-1
-255	rev3-2
- 10	rev3-3
-278	rev3-4
-299	rev3-5
-305	rev3-6
-340	rev3-7
-345	rev3-8
-497	rev3-9
-541	rev3-10
-466	rev3-11
-546	rev3-12
-542	rev3-13
-308	rev3-14

higher frequency of mutation. The difference in induction frequencies can also be accounted for by the slightly more liberal initial screening procedure used in the second experiment. In the search for new rev loci, replicas exhibiting reduced numbers of revertants were considered in addition to those having none at all.

It appears that the number of genes controlling UV-induced reversion in yeast is quite small. Only three genes were found in this study. In contrast, Cox and Parry (14), in an attempt to estimate the number of genes controlling UV-sensitivity in yeast, found 96 UV-sensitive mutants that occupied 22 different loci, but UV-induced mutation was not measured. The rev genes also have the UV-sensitive phenotype. Thus, most UV-sensitive mutations leave the induced mutation phenotype unchanged or enhanced (2, 64, 79, 111), while very few result in a reduced mutational response to UV. In the present study, 12 UV-sensitive mutants exhibiting reversion ability were isolated and found to represent 11 complementation groups.

It is possible to assert that many of the rev alleles, especially rev3 alleles, are not in fact different alleles of the same gene, but exact copies of the same allele which arose by cell division during or after the mutagen treatment. This is unlikely since the conditions of EMS treatment were not favorable to growth. In addition, the ratio of numbers of rev1 : rev2 : rev3 of alleles isolated in the two separate experiments was about the same, i.e., 1 : 1 : 3 for the first and 1 : 1 : 5.5 for the second experiment. The twenty rev

mutants probably represent different alleles.

Of the rev genes, rev3 was induced with a relative frequency almost five times that of either rev1 or rev2. This could occur, for example, if the rev3 gene was about five times as large as either rev1 or rev2. Esposito (18) and Jones (38) describe experiments in which the mutation rate corresponded to the length of the gene, determined independently by inter-allelic mapping. Theoretically, this hypothesis concerning rev3 could be tested directly using inter-allelic X-ray mitotic mapping (54), but such a measurement is not feasible at this time because of the phenotype associated with rev.

Meoitic mapping, however, offers a brighter prospect since meiotic recombination occurs at a higher frequency than mitotic recombination. After sporulation of heteroallelic rev diploids, clones developed from random spores could be tested for their UV-reversion ability. Such reverting ascosporal clones could arise as a result of a reciprocal exchange between the alleles or by gene conversion of one of the alleles to wild-type. The frequency of UV-reverting spore clones could then be used as a relative measure of the distance between rev allele pairs.

B. UV-induced reversion

1. Haploid Strains. In Figure 3 the frequency of locus reversion of arg4-17 is plotted versus UV dose for 5 rev segregants:

X1687-16C	REV	arg4-17
XY6-5A	rev1-1	arg4-17
XY36-3D	rev2-1	arg4-17
XY19-1D	rev3-1	arg4-17
XY82-7B	rev3-2	arg4-17
XY84-3C	rev3-3	arg4-17

At a dose of 265 erg/mm^2 in the wild-type 109 revertants per 10^7 survivors are produced, while the corresponding frequencies in the rev1-1, rev2-1, rev3-1, rev3-2 and rev3-3 strains are about 3, 30, 3, 30 and 12, respectively. Figure 4 shows the frequency of induced suppressors plotted versus UV dose for the same strains. The frequency of induced suppressors was calculated by subtracting the spontaneous value from the suppressor frequency at each dose.

These results quantitatively describe the reversionless phenotype on which the selection of rev mutants was based. The frequencies of UV-induced locus reversion of arg4-17 at 265 erg/mm^2 in these 5 rev strains range between about 1/30 and 1/3 that of the wild-type. The rev3 alleles result in different reversion phenotypes, spanning the entire range given above.

Suppressor mutants of arg4-17 are induced by low UV doses in the rev1-1, rev2-1, and rev3-1 strains at rates equal to or less than that in the wild-type. The rev3-2 and rev3-3 strains have greater induced suppressor frequencies. If suppressors arise from addition-deletion events, as suggested by Magni et al. (53, 53a), the rev3-2 and rev3-3 alleles may block the repair of such premutational damage which in turn is eventually converted into frameshift mutations by

Figure 3. Locus reversion of arg4-17 vs. UV dose in REV and rev haploid strains.

Figure 4. Induced suppressor reversion of arg4-17 vs. UV dose in REV and rev haploid strains. Frequencies were calculated by subtracting the zero-dose frequency from the frequency at each dose.

Figure 3

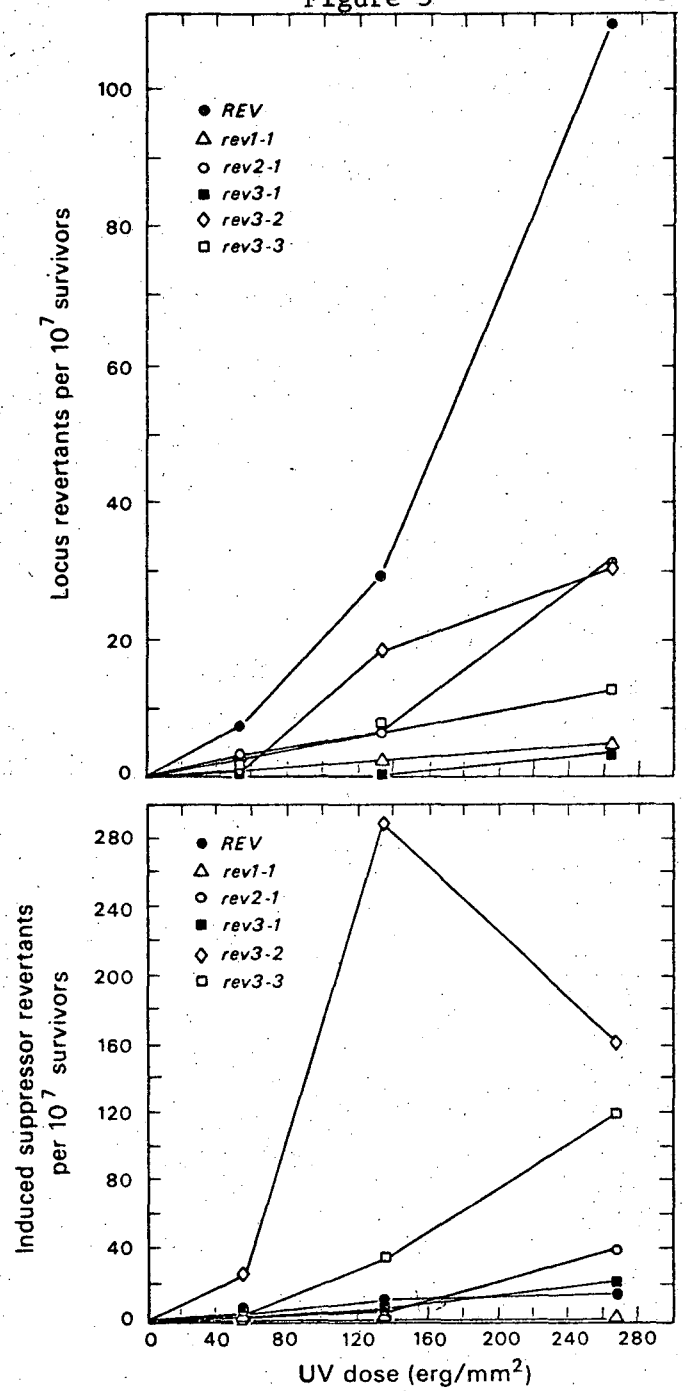

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Figure 4

another pathway.

In both rev and REV strains the spontaneous arginine revertants were due almost entirely to suppressors. In the REV strain suppressors are induced at a rate far below the rate of induction of locus revertants. That is, most of the total UV-induced revertants are locus revertants. This was also observed by Resnick (79). In rev strains, however, since locus reversion is significantly reduced, a large proportion of the total UV-induced revertants is due to the induction of suppressors.

2. Diploid strains homozygous for rev genes.

Diploid strains homozygous for the three rev genes were constructed by crossing haploid segregants carrying the same rev allele. The following strains were used:

XY129	REV/REV	arg4-17/arg4-17
XY150	rev1-1/rev1-1	arg4-17/arg4-17
XY127	rev2-1/rev2-1	arg4-17/arg4-17
XY186	rev3-1/rev3-1	arg4-17/arg4-17

The rev3-2 and rev3-3 alleles were not studied. The induction curves for the locus reversion of arg4-17 in these four diploid strains are shown in Figure 5. As in the haploid case, the rev3-1/rev3-1 strain is most severely reduced in locus reversion induction, with the rev1-1/rev1-1 strain intermediate, and the rev2-1/rev2-1 strain least affected.

Figure 6 shows the frequencies of induced suppressor reversion of arg4-17 in these strains versus UV dose. As in the

Figure 5. Locus reversion of arg4-17 vs. UV dose in REV/REV and rev/rev diploid strains.

Figure 6. Induced suppressor reversion of arg4-17 vs. UV dose in REV/REV and rev/rev diploid strains. Frequencies were calculated by subtracting the zero-dose frequency from the frequency at each dose.

Figure 5

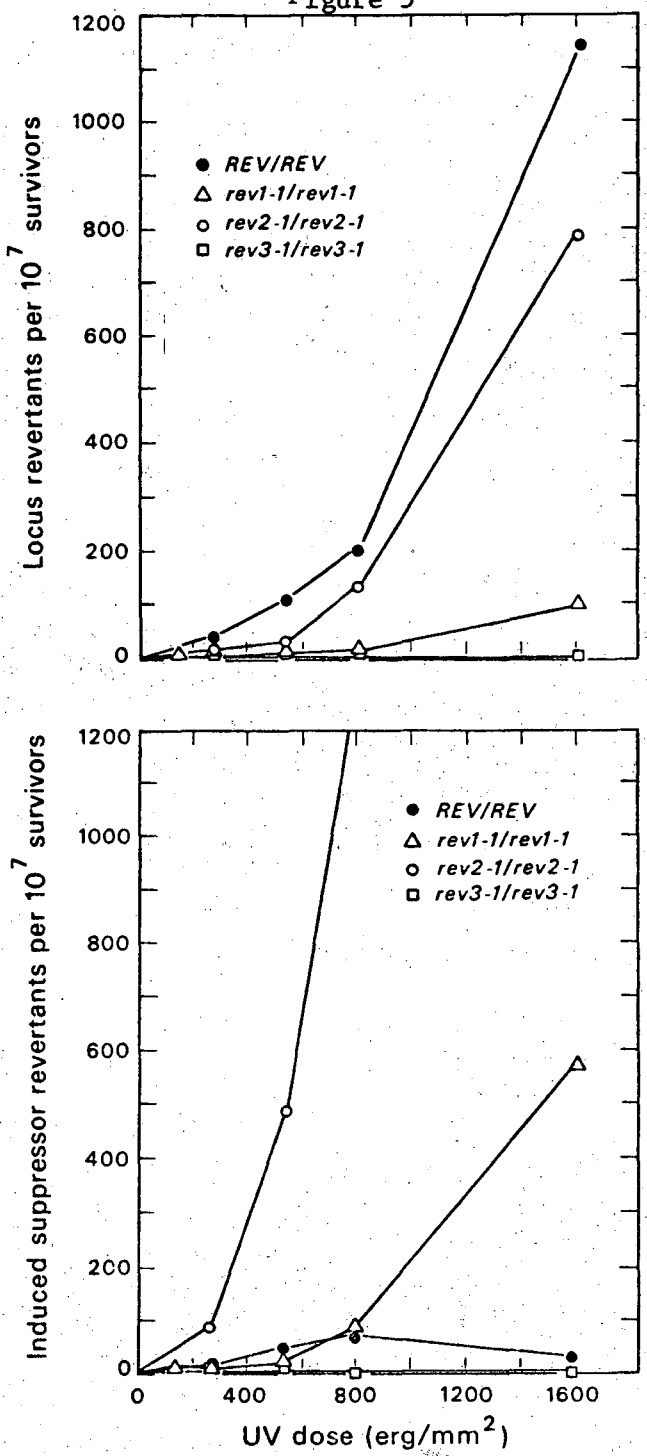

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Figure 6

haploid, the induction of locus revertants in the REV/REV diploid is far greater than that of suppressor revertants. The UV doses employed in the measurement of UV-reversion and UV-survival in diploid strains were much greater than those used in the haploid. In the low dose region, the rev3-1/rev3-1 strain is severely blocked in suppressor induction and the rev1-1/rev1-1 strain appears to have a similar suppressor induction response as the control. The rev2-1/rev2-1 strain, however, appears to have an enhanced suppressor induction.

3. Diploid strains heterozygous for rev genes. Figure 7 shows the curves of UV-induction of arg4-17 locus revertants in the following strains:

XY63	REV/REV	arg4-17/arg4-17
XY6	rev1-1/REV	arg4-17/arg4-17
XY64	rev1-1/rev1-1	arg4-17/arg4-17

The strain heterozygous for rev1-1 exhibits an induction curve nearly equal to that of the REV/REV strain. The rev1-1/rev1-1 homozygote is severely reduced in UV-induced reversion of arg4-17. The same effect was seen with respect to UV-survival. This shows that rev1-1 is recessive to REV1 with respect to both the reversionless and the UV-sensitive phenotypes. Diploids carrying rev2-1/REV and rev3-1/REV were also found to exhibit UV-survival and UV-reversion phenotypes similar to those expressed by REV/REV diploids. Thus, both rev2-1 and rev3-1 were also considered to be recessive to their wild-type forms. The complementation pattern obtained for reversionless mutants (Figure 2) supports this conclusion.

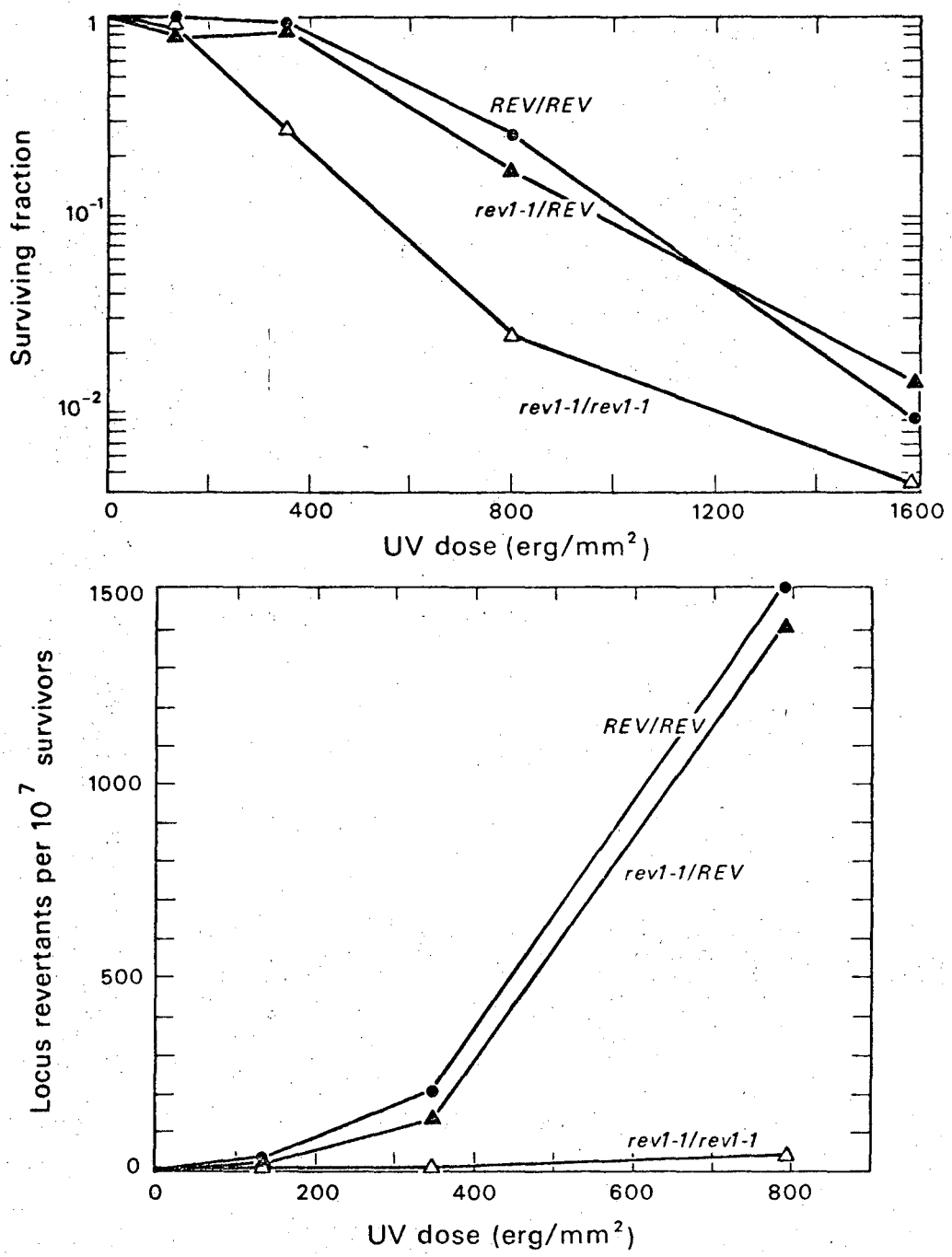

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Figure 7.--UV-survival curves (A) and locus reversion of arg4-17 vs. UV dose (B) in REV/REV, rev1-1/REV and rev1-1/rev1-1 diploid strains.

C. UV-sensitivity

1. Haploid strains. The three rev genes cause cells to be moderately UV-sensitive. The UV-survival curves of the haploid rev strains discussed previously are shown in Figure 8. Cells carrying rev3-1 are more UV-sensitive than cells carrying either rev1-1 or rev2-1. Further, all three strains carrying a single rev3 allele appear to be about equally UV-sensitive. The Dose-Reduction Factor (DRF) at S% survival of one strain compared to a reference strain is defined as the dose required to reduce the survival of the reference strain to S% divided by the dose required to reduce the survival of the strain in question to S%. The DRF's at 40% survival for these rev strains compared to wild-type are 3.7, 4.3, 8.5, 8.5 and 8.5 for the rev1-1, rev2-1, rev3-1, rev3-2 and rev3-3 strains, respectively.

If at equal survival levels the numbers of unrepaired lethal lesions are equal, assuming that defective repair of lethal lesions is responsible for UV-sensitivity, then the DRF gives a measure of the amount of lethal damage that is repaired in the wild-type strain relative to the sensitive strain. That is, the rev1-1 strain repairs 27% of the lethal UV damage normally repaired by the wild-type. The rev2-1 strain repairs 23% and the rev3-1, rev3-2 and rev3-3 strains only about 12% of the lethal UV damage normally repaired by the wild-type. Unfortunately, the nature of the specific defective

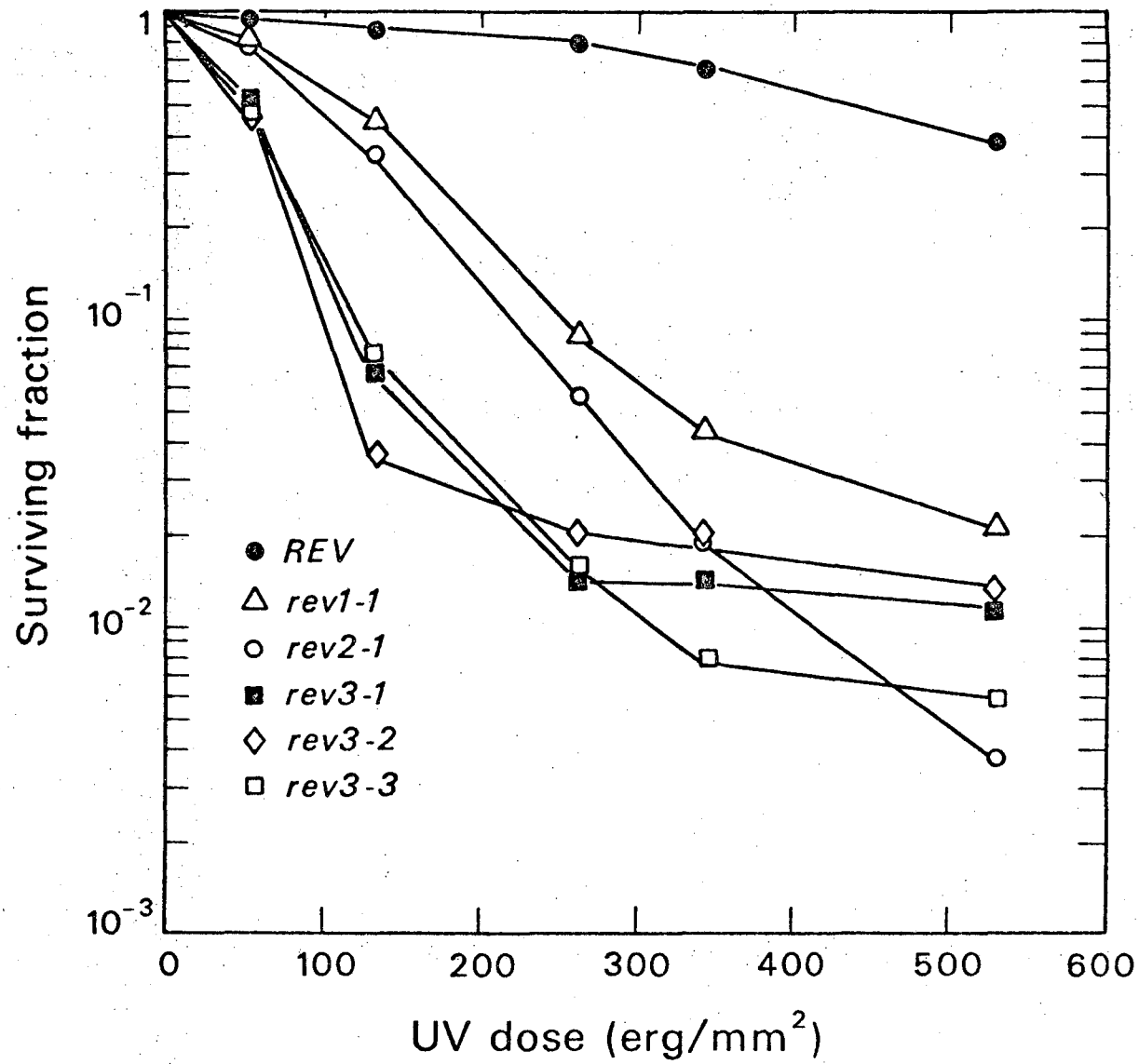

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Figure 8.--UV survival curves of REV and rev haploid strains.

repair is yet unknown. The UV-sensitivity of rev strains could be caused by defective excision repair or defective post-replication repair. It has not been demonstrated biochemically, however, that either of these procedures are operating in yeast as they are in bacteria. Of course, there may be repair systems in yeast which are not found in bacteria.

In E. coli B excision defective mutants (hcr) have DRF's of about 20, while exr mutants have DRF's of only about two or three (106), compared to wild-type. The hcr mutants produce many mutations among the survivors after UV exposure (29, 105). These mutations result from the large numbers of pyrimidine dimers which are left unexcised and which eventually are converted into mutations, presumably by the post-replication repair system (107). On the other hand, no UV-induced mutations are produced in exr strains (106). The rev mutants in yeast appear to be similar to the exr mutants of E. coli in that both are UV-sensitive to a moderate degree (compared to other extremely UV-sensitive mutants in E. coli and in yeast) and both are defective in UV-induced mutation.

2. Diploid strains. UV-survival curves were obtained for the following diploid strains:

XY129	REV/REV
XY150	rev1-1/rev1-1
XY127	rev2-1/rev2-1
XY128	rev3-1/rev3-1
XY186	rev3-1/rev3-1

The curves are shown in Figure 9. In contrast to the haploid, the rev3-1/rev3-1 diploid is not twice as UV-sensitive as either of the other two homozygous rev/rev diploids. All rev/rev diploids appear to exhibit approximately the same UV-sensitivity. The DRF at 10% survival is about 2, compared to wild-type. The meaning of the resistant tail on the curve for XY186 at higher UV doses was not investigated in this study. The rev3 haploids were also found to be susceptable to this tail effect (Figure 8).

D. Segregation of the rev phenotypes

Both the UV-reversionless phenotype and the UV-sensitive phenotype segregate together after sporulation of rev/REV diploids and are hence different phenotypic expressions of a mutation affecting single rev genes. These genes segregate in a regular 2 : 2 fashion during meiosis. This was a primary criterion in the selection of reversionless mutants. In a single ascus two spores are UV-resistant like the wild-type and have reversion ability, while the other two spores are UV-sensitive and lack reversion ability. To illustrate this point, each of the four spores from a single ascus, produced by a rev1-1/REV diploid, was subjected to a quantitative test of UV-survival and UV-reversion of arg4-17. The following spores were cultured and tested:

XY6-5A	rev1-1	arg4-17
XY6-5B	REV	arg4-17
XY6-5C	REV	arg4-17
XY6-5D	rev1-1	arg4-17

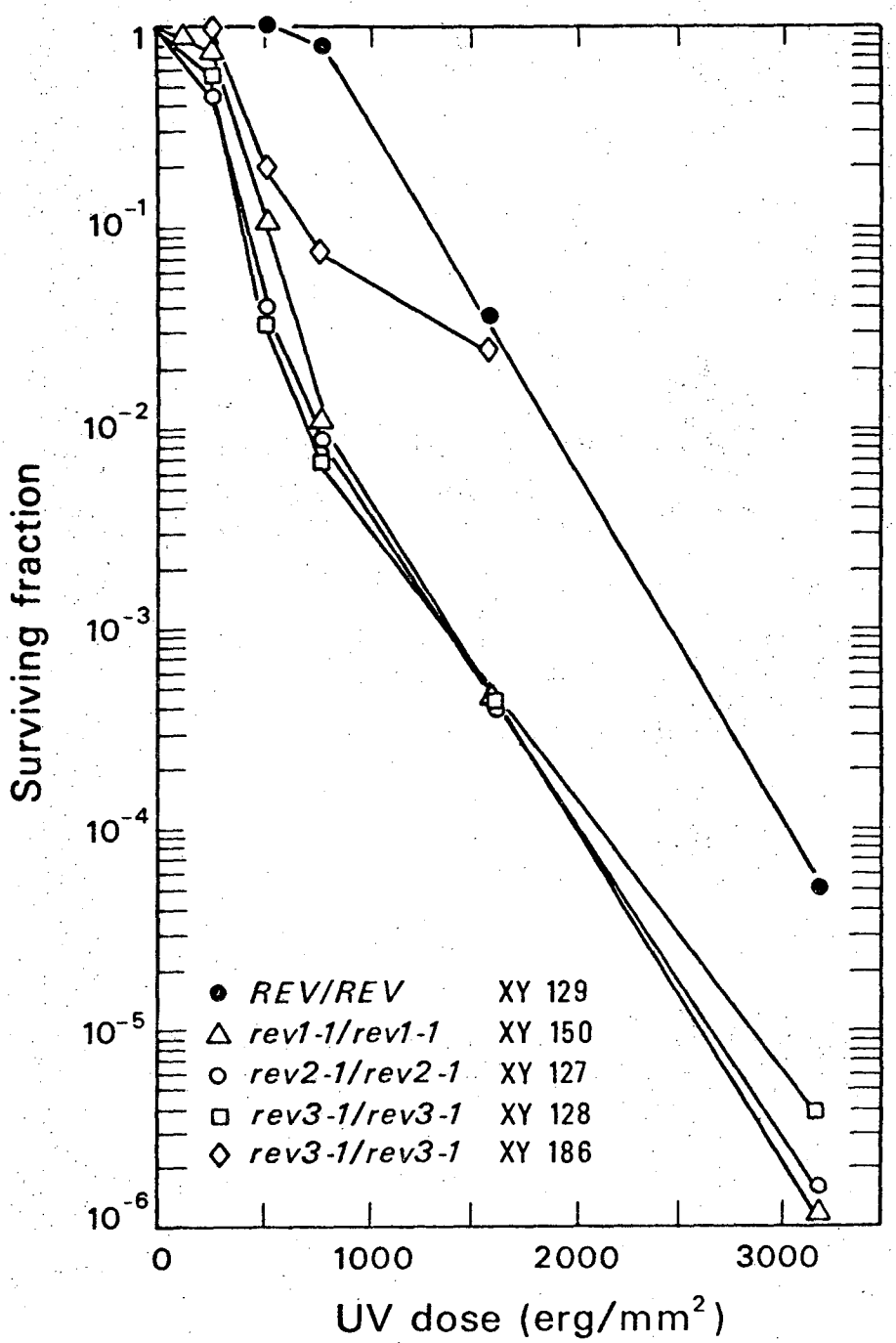

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Figure 9.--UV-survival curves of REV/REV and rev/rev diploid strains.

The curves shown in Figure 10 indicate that the UV-sensitive phenotype segregates with the UV-reversionless phenotype. Similarly, it was found that every meiotic segregant bearing either rev2-1 or rev3-1 is also both UV-sensitive and UV-reversionless.

E. X-Ray sensitivity

All three rev genes cause haploid cells to be only slightly X-ray sensitive. Quantitative comparisons of X-ray sensitivity in rev haploids compared to wild-type was difficult because of the characteristic tail on the survival curve at higher doses. This effect is due to the presence of budding cells in the irradiated population. Such budding cells are more X-ray resistant than non-budding cells (4). Comparisons of X-ray sensitivity were made in diploid strains homozygous for rev genes, relative to the wild-type diploid.

Diploids are in general more resistant to the lethal effects of both UV and X-ray irradiation than haploids. The X-ray survival curves of the three different rev/rev diploids XY150, XY127, XY128 and the wild-type diploid XY129 are shown in Figure 11. The rev1-1/rev1-1 strain is the most X-ray sensitive with a DRF at 10% survival of 2.4. The rev2-1/rev2-1 and the rev3-1/rev3-1 diploids are less X-ray sensitive each with a DRF of 1.5 and 1.8, respectively. As in the case of the UV-sensitive phenotype, the X-ray sensitive phenotype segregates with the reversionless phenotype in REV/rev crosses and is thus another expression of a single rev

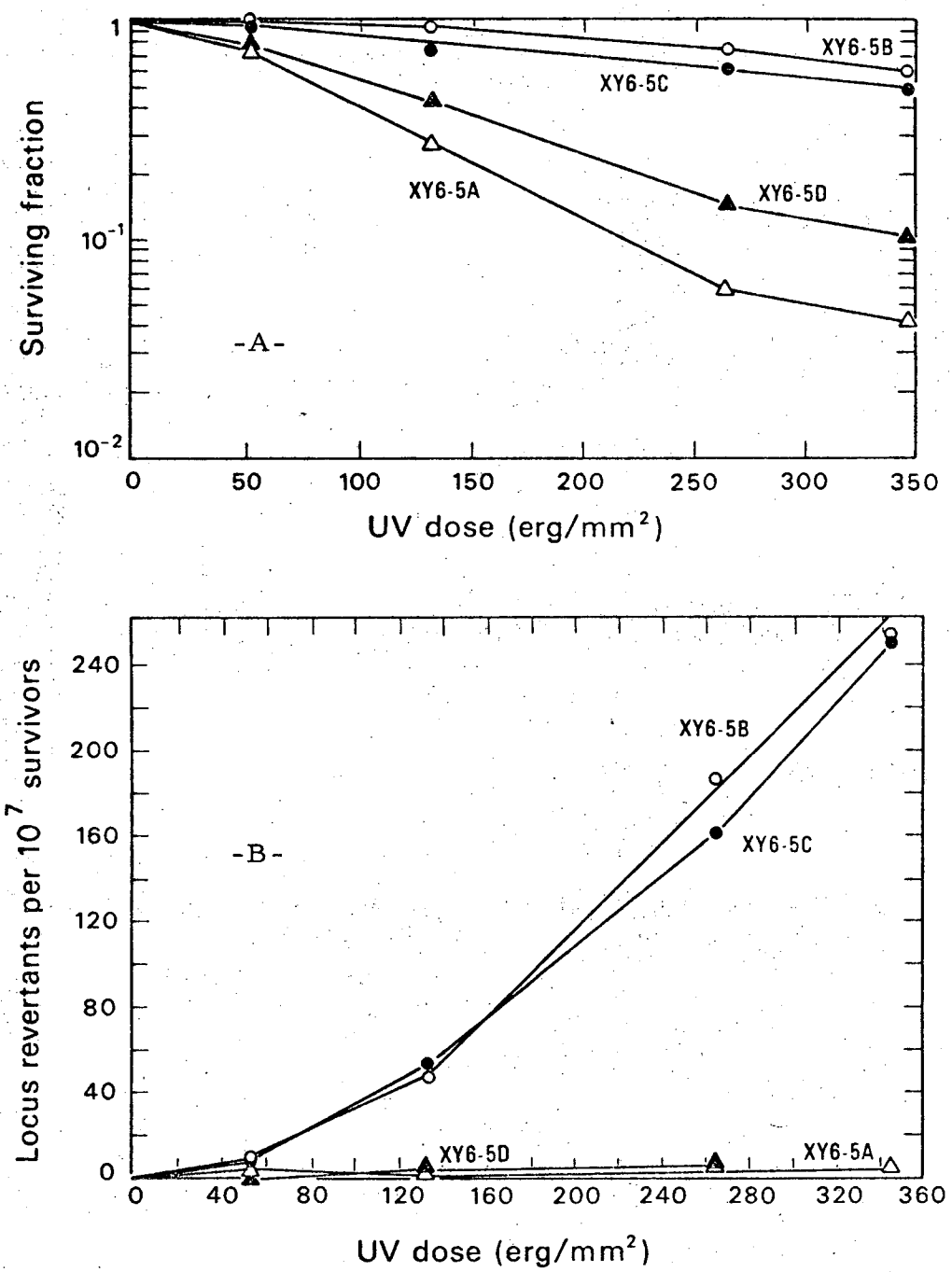

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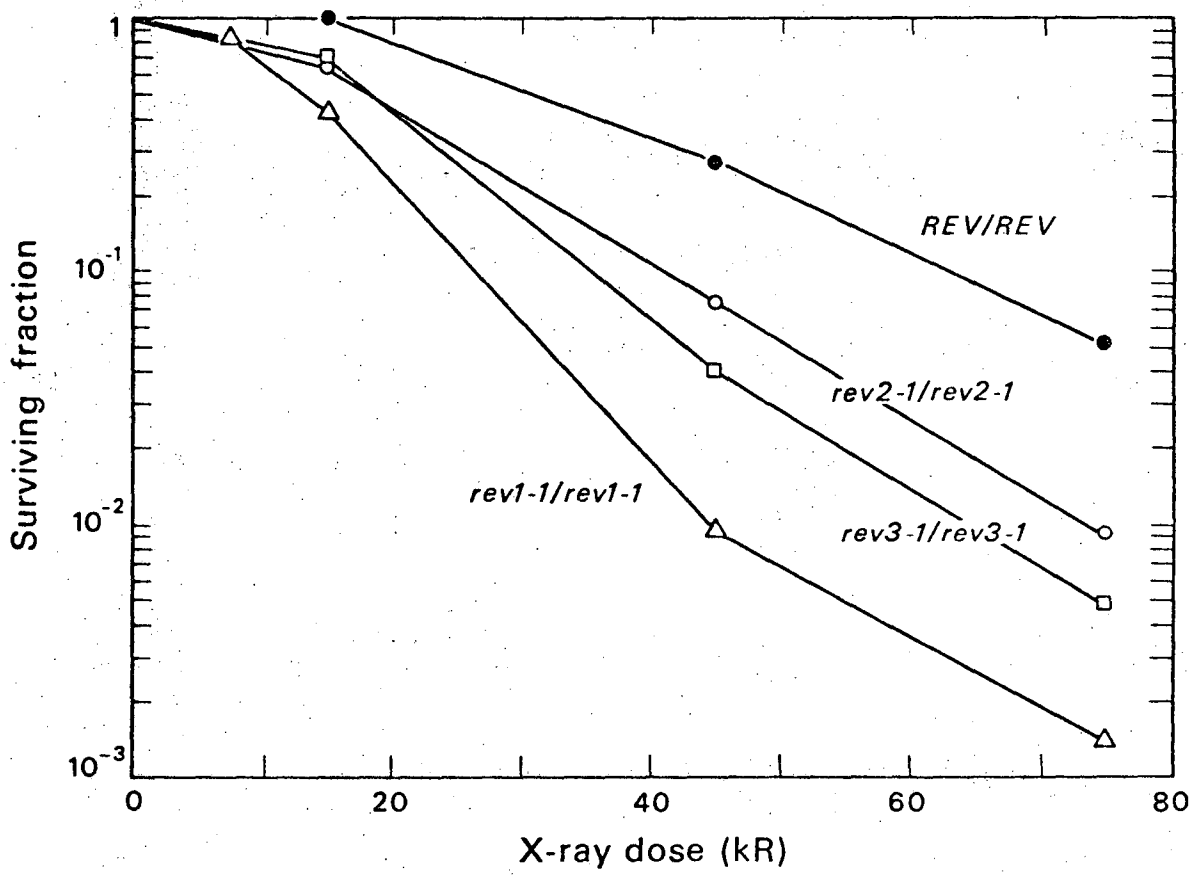
Figure 10. --UV-survival curves (A) and locus reversion of arg4-17 vs. UV dose (B) in four haploid meiotic segregants of a rev1-1/REV diploid.



DBL 708 5847

Figure 11.--X-ray survival curves of REV/REV and rev/rev diploid strains.

mutation. This suggests the following: that rev genes may act in one or more pathways of the repair of lethal UV-damage; that these pathways, when operating in REV strains, also produce UV-induced mutations among the survivors; and that the pathways of repair of lethal X-ray damage may share some common steps with those of the repair of UV-damage. The exr mutants of E. coli are also X-ray sensitive, as well as UV-sensitive (34, 81). That rev mutants are both moderately UV and X-ray sensitive is consistent with the hypothesis that they lack functions analogous to the exr+ function in bacteria.

F. EMS-sensitivity

The alkylating agent ethylmethanesulfonate (EMS) is both lethal and mutagenic to cells. In bacteria the lethal action of monofunctional alkylating agents such as EMS is attributed to single-strand breaks in DNA resulting from hydrolysis of triester phosphates (1) or following depurination (9). X-rays are also thought to produce single-strand breaks along the parental backbone of DNA (19, 39, 57). If EMS-induced lethal damage in yeast is repaired by pathways not involving the repair of UV and X-ray lethal damage, then rev strains would be expected to show the same sensitivity to killing as that exhibited by the wild-type. If some common steps are shared, then one or more of the rev genes may cause cells to be also EMS-sensitive.

The following strains were tested for sensitivity to EMS as

measured by colony survival after varying exposure times:

XY129-2A	REV
XY14-28A	rev1-1
XY36-8A	rev2-1
XY38-3D	rev3-1

The procedure followed was the same as that employed in the original induction of rev mutants as described in Materials and Methods (51), except that the cells were incubated in 3% EMS for varying lengths of time instead of one hour. The survival curves are shown in Figure 12.

The rev2-1 and rev3-1 strains each exhibit approximately the same EMS-sensitivity and are much more EMS-sensitive than the REV strain. The dose reduction factor at 1% survival is 2.7. The rev1-1 strain, by contrast, appears to be as EMS-sensitive as the wild-type as measured by the slopes of the survival curves, but more EMS-sensitive than control (DRF of 1.1) when compared on the basis of survival alone because the shoulder is somewhat smaller than the shoulder exhibited by the wild-type. These results suggest that rev2-1 and rev3-1 (and perhaps rev1-1) may block the repair of EMS-induced lethal damage. The case of EMS-induced mutation will be considered later.

G. Mapping of rev genes

Systematic attempts to localize new genes to a particular linkage group usually involves a determination of centromere-linkage as a first step. Tetrad data for each of the rev genes revealed that only rev2 was linked to a centromere. The centromere-linked

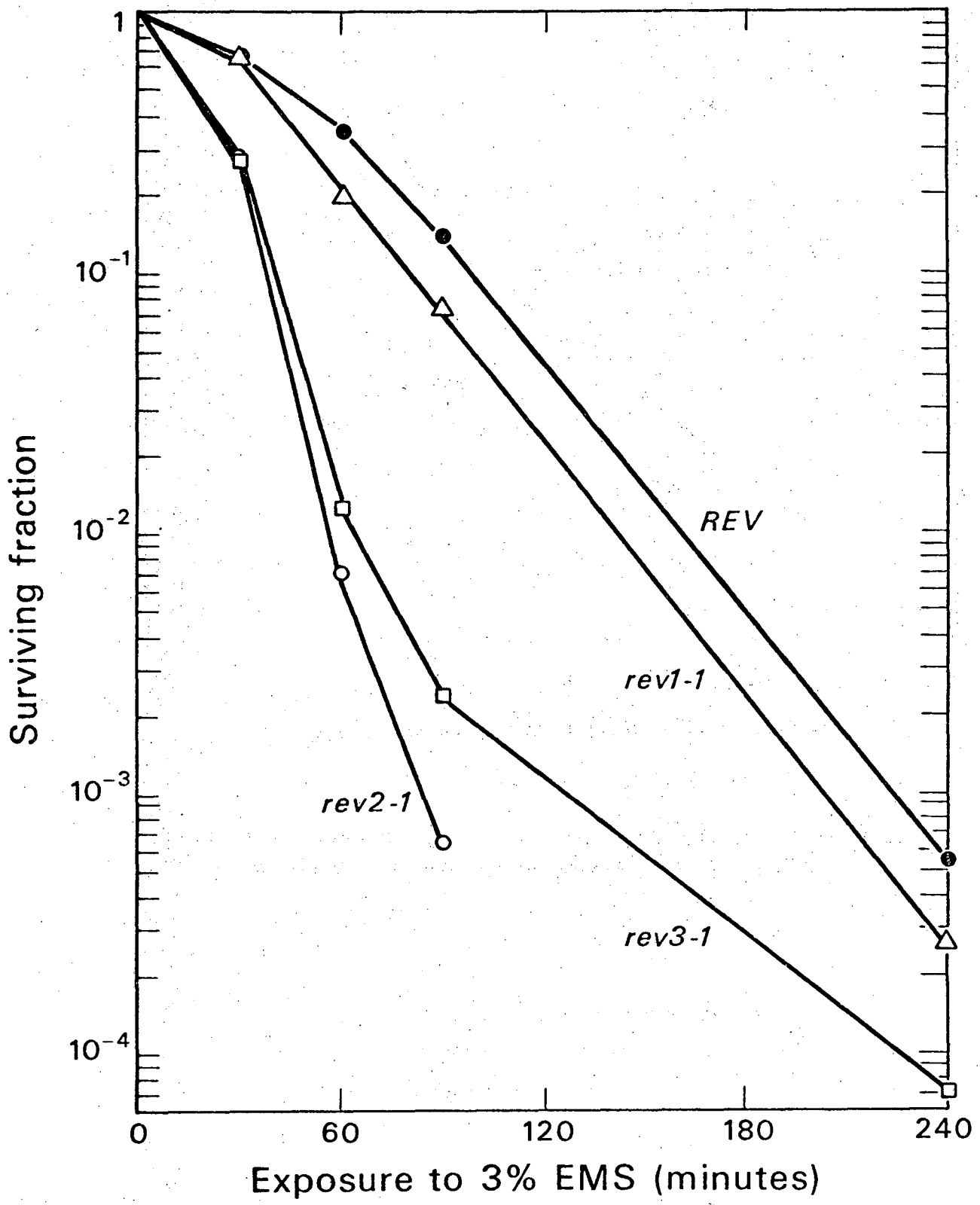


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Figure 12. --Sensitivity of colony-formation to treatment with 3% EMS in REV and rev haploid strains.

marker leu1-12, which has a SDS frequency of about 5% (61), was used to indicate centromere-linkage. The pooled data for the numbers of PD, NPD and T asci are given in Table 3. The gene pair rev2-leu1 exhibits a tetratype frequency significantly less than 2/3.

In order to determine the particular centromere to which rev2 was linked, a strain carrying rev2-1 was then crossed to a series of strains that carried markers located near the centromeres of different linkage groups. The numbers of PD, NPD and T asci for various gene combinations involving rev2 are shown in Table 4. The data indicate that rev2 is linked to asp5 which is near the centromere of linkage group XII. The distance x in centimorgans (cM) between the two genes can be calculated from the equation (72):

$$x = 50(T + 6NPD) / (PD + NPD + T)$$

For PD : NPD : T values of 80 : 0 : 7 for the gene pair rev2-asp5, the distance between rev2 and asp5 is calculated as about 4.0 cM.

In the cross of rev2 to asp5, the trp1 gene was also segregating. The marker trp1 is tightly linked to its centromere, i.e., SDS frequency = 0.94% (61). Thus, observation of the spore array of asp5 compared to that of trp1 can be used to detect cross-over events between asp5 and its centromere. It was found that in each of the seven asci with an exchange between rev2 and asp5, asp5 showed first division segregation. Thus, the most likely arrangement of the two genes on chromosome XII is centromere - asp5-rev2.

Table 3.--Numbers of PD, NPD And T asci in crosses involving rev and leu1

Gene Pair	PD*	NPD	T	%T	SDS Frequency
rev1-leu1	15	11	53	67.0	0.670
rev2-leu1	17	31	26	35.1	0.324
rev3-leu1	11	16	51	65.4	0.654

*Pooled data.

Table 4.--Numbers of PD, NPD and T asci in crosses involving rev2 and various centromere-linked genes.

Chromosome number	Centromere-linked gene	PD	NPD	T	%T
1	*ade1*	3	3	1	10.0
2	*gal1*	11	11	9	29.0
3	α	5	4	21	70.0
4	*trp1*	13	12	7	21.9
5	*ura3*	9	12	11	34.4
6	*his2*	8	5	14	51.8
7	*leu1*	17	31	26	35.1
8	*arg4*	9	11	11	35.5
9	*his6*	7	5	5	29.4
10	*iso3*	4	3	5	41.7
11	*met14*	12	11	6	20.7
12	*asp5*	80	0	7	8.05
13	*lys7*	11	6	12	41.4
14	*pet8*	4	11	4	21.0
15	*pet17*	0	2	6	75.0
16	*tyr7*	0	3	5	62.5
17	*uvs9*	3	4	12	63.2

DBL 708 5858

From Table 3 rev2 segregates at the second division in 32.4% of the tetrads. If double exchanges in the centromere-rev2 interval are assumed to be rare, then the distance of rev2 from its centromere can be approximated as one-half the SDS frequency, or 16.2 cM. Mortimer and Hawthorne (61) have estimated the distance from asp5 to its centromere to be 13.6cM. Thus, the predicted distance between rev2 and the centromere is 13.6 cM plus 4.0 cM or 17.6 cM, assuming that the distances may be summed. The observed value of 16.2 cM is not significantly different from 17.6 cM.

H. Specificity of the induced-mutation phenotype of rev genes

The rev genes have been shown to interfere with UV-induced locus reversion of arg4-17. In studying the mechanism of action of these genes, it is essential to know more about their specificity. Strains carrying rev genes were tested for their ability to interfere with induced mutagenesis as measured by the following: UV-induced reversion of a missense allele (arg4-6); UV-induced forward mutation to the petite phenotype; UV-induced and EMS-induced forward mutation to ade1 or ade2.

1. UV-induced reversion of an ochre-suppressible allele: lys1-1. The lys1-1 allele can be suppressed by the same ochre-suppressors that suppress arg4-17. Presumably both arg4-17 and lys1-1 determine the same nonsense codon UAA (20, 26, 28). The lys1 gene segregates independently of arg4 (61) and therefore

offers an opportunity to obtain evidence about the specificity of rev genes. Site-specific rev genes should also reduce the UV-reversion of lys1-1, while locus specific rev genes, however, would not.

The following strains were tested for UV-induced locus reversion of lys1-1:

X1687-16C	REV	lys1-1
XY6-5A	rev1-1	lys1-1
XY36-3D	rev2-1	lys1-1
XY19-1D	rev3-1	lys1-1

The induction curves are shown in Figure 13. The UV-survival curves of these strains are shown in Figure 8. All three rev alleles block, to varying degrees, the induction of site revertants of lys1-1. At a UV dose of 265 erg/mm^2 the REV strain produces about 86 revertants per 10^7 survivors, whereas the rev2-1 strain produces about 53, the rev1-1 strain about 10, and the rev3-1 strain about 7. Comparing these curves to the induction curves for locus revertants of arg4-17 in the same strains (Figure 3), it can be seen that in both instances, rev2-1 has the least effect in reducing reversion, while rev1-1 and rev3-1 have a much greater effect.

In the high dose region the induction frequencies of lys1-1 locus revertants (Figure 13) are slightly lower than the frequencies of arg4-17 reversion (Figure 3), when measured in the REV strain. A possible reason for this is given in discussion.

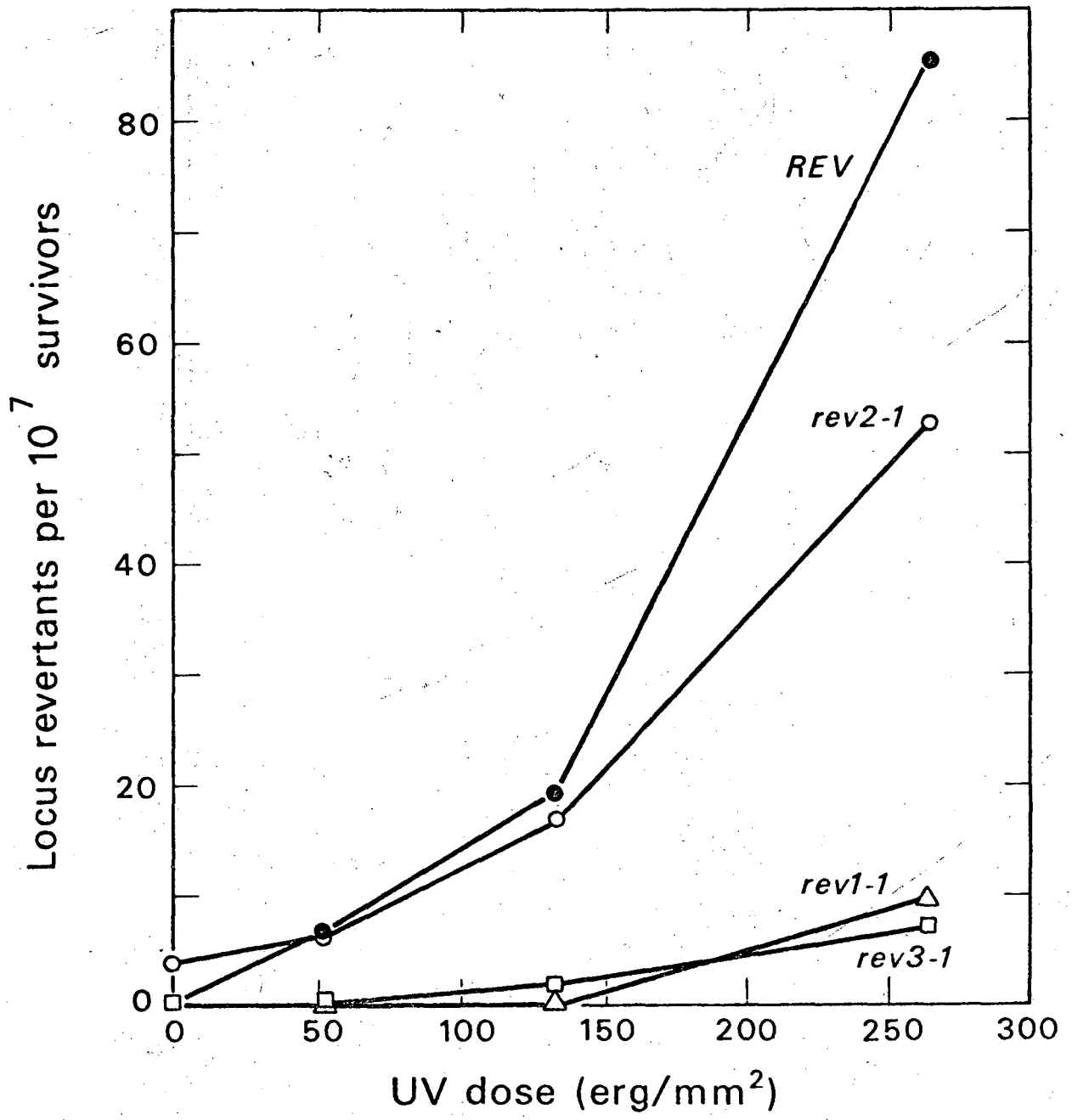

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Figure 13.--Locus reversion of lys1-1 vs. UV dose in REV and rev haploid strains.

2. UV-induced reversion of a missense allele: arg4-6. The osmotic remedial arg4-6 allele is not suppressible by ochre-suppressors, and it complements other arg4 alleles (59). On this basis, arg4-6 is probably a missense mutation. It is also UV-revertible (59, 48), but the induced reversion frequencies are not as great as found for arg4-17.

To determine if rev genes can interfere with UV-induced reversion of missense as well as nonsense alleles, UV-induced reversion of arg4-6 was measured in rev strains and compared to wild-type. This was achieved by constructing strains each of which carried both arg4-6 and rev. To do this, rev haploids carrying arg4-17 were crossed to strains carrying arg4-6. After sporulation the spores were dissected and identified by crossing each spore to two tester strains, one carrying arg4-6 and one carrying arg4-17. These test diploids were replica-plated to C-AR and then exposed to an X-ray dose of 10 Kr which is sufficient to induce an observable number of prototrophs in arg4-6/arg4-17 heteroallelic diploid replicas by means of mitotic recombination. The arg4-6 segregants, when crossed to arg4-6 testers, form diploids that exhibit no such X-ray response. Similarly, the arg4-17 segregants, when crossed to arg4-17 tester strains, form diploids that exhibit no X-ray induced prototrophs. The segregation of the rev gene was detected by analyzing the UV-reversion response of arg4-17 segregants. The rev arg4-6 segregants were deduced. The following segregants were identified

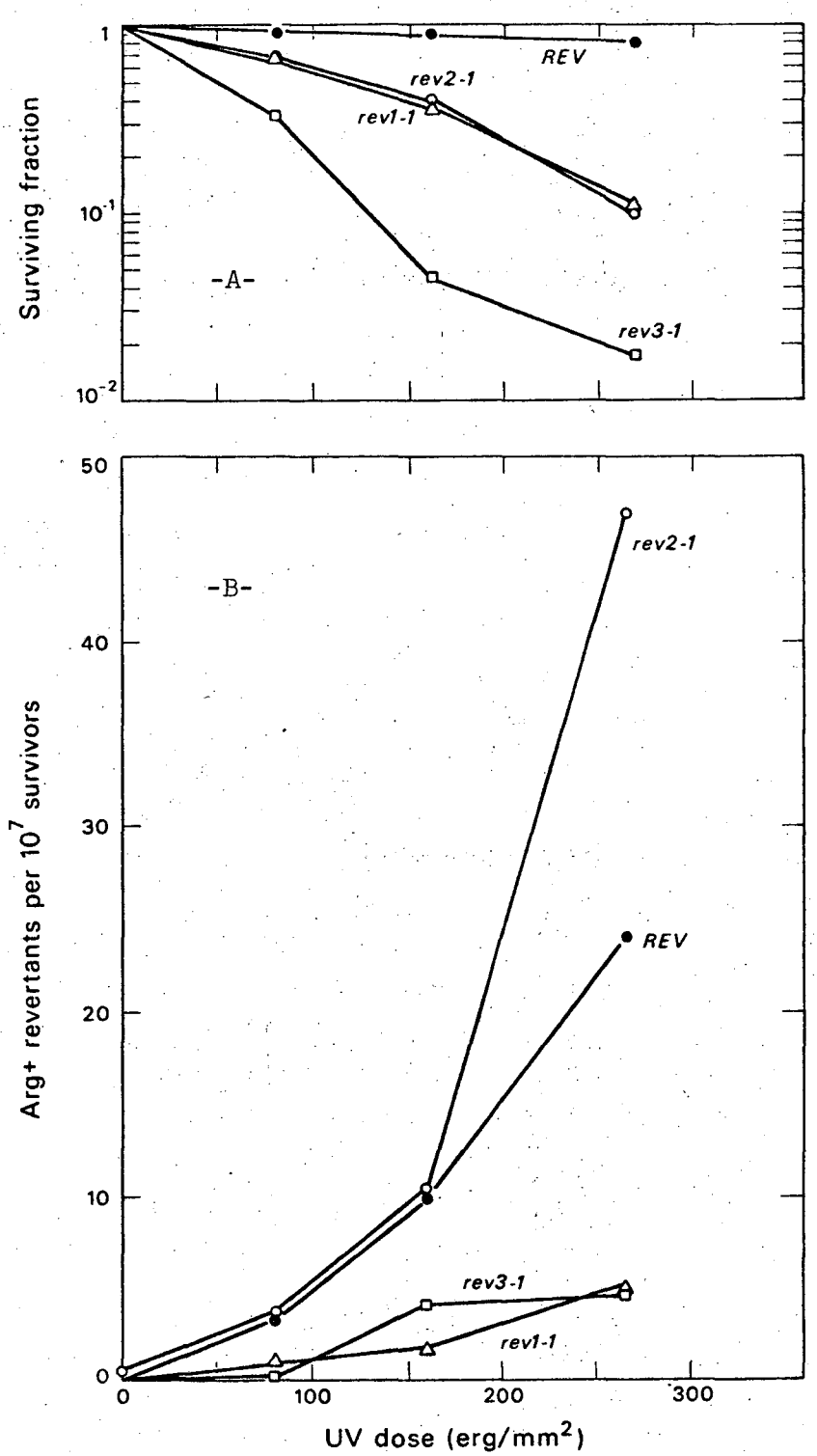
by this method:

XY134-4A	REV	arg4-6
XY133-2C	rev1-1	arg4-6
XY134-3C	rev2-1	arg4-6
XY135-5D	rev3-1	arg4-6

These strains were tested for UV-survival and reversion induction of arg4-6. Revertants of arg4-6 were considered to arise by reversion at the locus. It was not determined whether any revertants carried suppressors. This could be done by crossing a number of revertants to a wild-type strain, sporulating, and examining the ARG:arg ratio in each tetrad. A ratio of 4:0 in each ascus would indicate a locus revertant. A suppressor revertant would yield, when crossed to wild-type, 3 types of asci. The PD, NPD and T asci would exhibit ARG:arg ratios of 4:0, 2:2, and 3:1, respectively.

The results of UV-survival and reversion induction in rev arg4-6 strains are shown in Figure 14. The three rev strains exhibit the approximate survival curves expected of rev segregants. The reversion induction curves reveal that while the rev1-1 and rev3-1 strains are significantly reduced in UV-reversion of arg4-6, the rev2-1 strain is unaffected and actually shows about twice the wild-type response at a UV dose of 265 erg/mm^2. These data indicate that rev1-1 and rev3-1 may both have a general non-specific action in reducing UV-induced reversion but that rev2-1 may be specific only for locus reversion of ochre-suppressible strains as described below.

Figure 14.--UV-survival curves (A) and reversion of arg4-6 vs. UV dose (B) in REV and rev haploid strains.


Surviving fraction
1
10^{-1}
10^{-2}
-A-
REV
rev2-1
rev1-1
rev3-1
Arg+ revertants per 10^7 survivors
50
40
30
20
10
0
-B-
rev2-1
REV
rev3-1
rev1-1
0
100
200
300
UV dose (erg/mm^2)

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3. Forward mutation to the petite phenotype. Under aerobic conditions cells exhibiting the petite phenotype grow much more slowly than grande cells and thus form small white colonies when plated on a complete agar medium. Petite cells, also called respiratory-deficient (RD), appear white compared to the cream color of respiratory-sufficient (RS) cells because they lack certain cytochromes and enzymes needed in respiratory metabolism (17, 89). Such RD cells can arise by both nuclear mutation and loss of a cytoplasmic factor (12, 17). Unlike RS cells, petites fail to grow on non-fermentable substrates such as glycerol. Such a mutational system may be used to study the general effect of rev genes on forward mutation. The following strains were used to study UV-induced petite production: XY129-2A, X1687-16C, and XY134-4A all carried REV; XY14-28A, XY6-5A, and XY133-2C all carried rev1-1; XY36-8A, XY36-3D, and XY134-3C all carried rev2-1; XY38-8D, XY19-1D, and XY135-5D all carried rev3-1. Single doses of UV were employed. Cells were plated onto YEPD at appropriate dilutions and subsequently exposed to a dose of either zero or 265 erg/mm^2. After incubation the plates containing the viable clones were replica-plated to petite agar. Replicas failing to grow on petite agar were classified as having the petite phenotype. Petite mutants were also classed as either whole colony mutants or sectored mutants.

The results are shown in Table 5. The frequencies of induced petites in the three rev1-1 strains do not appear to be significantly different from the wild-type frequencies. The induced frequencies in the rev2-1 strains appear lower than wild-type in two strains (XY36-8A and XY36-3D) but about the same in the third strain (XY134-3C). The induced frequencies in all three rev3-1 strains are considerably higher than wild-type. All frequencies of induced petites were calculated by subtracting the spontaneous frequency from the total frequency.

Raut (74) found both genic and cytoplasmic petites after exposure to UV. Zakharov et al. (111) studied the production of petites by UV in both UVS and uvs strains and found that nearly all of the UV-induced petites contained the cytoplasmic factor. It was found that at equal UV doses the frequencies of petite induction were greater in the two UV-sensitive strains than in the wild-type strain. These UV-sensitive strains, however, were also more sensitive to UV-induced nuclear mutation. Zakharov et al. concluded that the repair system defective in the uvs strains is not localized in the nucleus and can also repair premutational damage in the cytoplasm, presumably in mitochondrial DNA (63, 103).

Assuming that the petites induced by UV in this study are cytoplasmic petites, it would appear that the rev3-1 strains are unable to repair premutational damage in the cytoplasm. Since these strains are blocked in the induction of nuclear mutations, the

Table 5.--Production of petites by UV in REV and rev strains

Strain	rev Genotype	UV Dose	% Survival	Total No. Surviving Clones	Total No. Petites			% Petites	
					Whole	Sector	Total	Total	Induced*
XY129-2A	REV	0	100	503	22	9	31	6.2	--
X1687-16C	REV	↓	↓	136	45	0	45	33.1	--
XY134-4A	REV			371	36	3	39	10.5	--
XY14-28A	rev1-1			795	56	5	61	7.7	--
XY6-5A	rev1-1			258	68	11	79	30.6	--
XY133-2C	rev1-1			349	29	0	29	8.3	--
XY36-8A	rev2-1			729	16	2	18	2.5	--
XY36-3D	rev2-1			159	83	14	87	54.8	--
XY134-3C	rev2-1			415	34	3	37	8.9	--
XY38-8D	rev3-1			719	25	0	25	3.5	--
XY19-1D	rev3-1			217	20	0	20	9.2	--
XY135-5D	rev3-1			300	59	0	59	19.7	--
XY129-2A	REV	265	71	579	75	20	95	16.4	10.2
X1687-16C	REV		77	151	47	14	61	40.4	7.3
XY134-4A	REV		80	249	34	13	47	18.9	8.4
XY14-28A	rev1-1	265	7.1	961	117	8	125	13.0	5.3
XY6-5A	rev1-1		13	339	117	15	232	39.0	8.4
XY138-2C	rev1-1		10	413	83	4	87	21.0	12.7
XY36-8A	rev2-1	265	12	1667	23	2	25	1.5	-1.0
XY36-3D	rev2-1		8.1	93	35	6	41	44.1	-10.7
XY134-3C	rev2-1		9.8	174	20	5	25	14.4	5.5
XY38-8D	rev3-1	265	1.5	357	126	7	133	37.3	33.8
XY19-1D	rev3-1		0.97	139	89	2	91	65.5	56.3
XY135-5D	rev3-1		1.7	321	180	2	182	58.0	38.3

*Computed by subtracting the spontaneous frequency from the total frequency.

mechanisms of mutation induction for nuclear and cytoplasmic mutations may be different. The rev1-1 strains may repair cytoplasmic premutational damage with the same efficiency as the REV strain, while rev2-1 strains may block the production of cytoplasmic mutations.

4. Forward mutation to ade1 or ade2. A sounder estimate for the induction of forward mutations than petite production is the selection of mutants defective at either the ade1 or the ade2 locus. The advantages of this system have been detailed by Roman (80). Mutants are restricted to only two nuclear loci. In the petite system, the total number of possible loci that can be induced is unknown and cytoplasmic petites are not excluded. Furthermore, the selection of petites depends upon replica-plating the clones surviving the mutagen treatment, while ade1 or ade2 mutant clones may be observed directly on the plate containing all survivors.

The presence of either of these two loci in cells will cause the accumulation of an intracellular red pigment that is easily seen in clones (75, 80). There are no other known mutations in yeast that will lead to a red-colored clone. The production of red pigment occurs only during aerobic metabolism (90). Thus, if a cell carries ade1 or ade2, but is also phenotypically petite, then no pigment will be produced. Auxotropic mutants at ade1 or ade2 can be selected directly by searching for red clones among the survivors. The frequency of red clones, however, must be based only on the

total number of RS survivors, since petites carrying ade1 or ade2 will not be scored as red clones. Therefore, the same strains must be assayed for the induction of petites by the same mutagen dose used for inducing red colonies. The frequency of reds among the total number of survivors is then divided by the RS fraction of the total to give the frequency of reds (per RS survivor). Strains carrying rev were tested for the induction of red clones by both UV and EMS. Single doses of each mutagen were used:

XY129-2A	REV	ADE
XY14-28A	rev1-1	ADE
XY36-8A	rev2-1	ADE
XY38-3D	rev3-1	ADE

The spontaneous frequencies of mutation for a similar system occuring in Schizosaccharomyces pombe have been estimated to be on the order of 10^{-7} or smaller (52). Therefore, only induced mutation frequencies were determined as shown in Table 6.

When UV was used the total number of red clones scored in each strain was low. The data were therefore pooled to obtain an estimate of the average induced mutation frequency in all three rev strains. The average mutation frequencies to ade1 or ade2 in rev strains is less than that in the REV strain. The small numbers of red clones make this difference not very significant. The standard error is estimated as 35% or 0.5×10^4 for wild-type and 41% or 0.15×10^4 for rev strains.

After an exposure of 60 minutes to EMS, all three rev strains, in contrast to the UV case, have significantly higher frequencies of

Table 6.--Forward mutation to ade1 or ade2 induced by UV and EMS in REV and rev strains

Strain	rev genotype	Dose	Average % survival	Total No. Surviving Clones	Total No. Red Clones	% RS	Frequency of Reds (per RS Survivor) x 10^4
		UV(erg/mm^2)					
XY129-2A	REV	265	71	68,060	8	83.6	1.4
XY14-28A	rev1-1	265	7.1	56,220	2	87.0	
XY36-8A	rev2-1	265	12	93,300	2	98.5	0.37*
XY38-3D	rev3-1	265	1.5	32,650	2	62.7	
		EMS(minutes)					
XY129-2A	REV	60	49	119,440	52	70.8	6.15
XY14-28A	rev1-1	60	42	172,400	252	78.3	18.7
XY36-8A	rev2-1	60	6.4	50,160	51	79.4	14.7
XT38-3D	rev3-1	60	4.2	239,800	348	66.6	21.8

*Average frequency for all rev strains calculated by dividing the total number of reds, 6, by the total number of RS survivors.

forward mutation than does the REV strain. That rev strains are EMS-sensitive (Figure 12) indicates that they may be involved in one or more pathways of repair of lethal EMS damage. Since more induced forward mutations are produced among the survivors in rev strains compared to the REV strain, the mutations induced in the REV strain are probably not produced by these repair pathways themselves. If one of these repair pathways is turned off by a rev mutation, then there will be more unrepaired EMS damage than before. This unrepaired EMS damage could then act as a substrate in a later pathway that does itself produce mutations.

I. Strains carrying two or more UV-sensitive genes

In studying the genetic control of metabolic pathways, it is essential to know whether genes controlling the formation of a product are acting in a single linear pathway, or whether they participate in a more complicated mechanism involving two or more independent pathways. One way to distinguish between these possibilities is to observe the response of a strain carrying two or more mutant genes relative to the response of the single mutant strains. If a single linear pathway is operative, such multiple mutant strains would be expected to exhibit the same phenotype as any one of the single mutant strains. If two or more parallel pathways are involved, strains multiply mutant for genes in different pathways would be expected to exhibit a response that is less than

any one of the single mutant strains.

1. Multiple rev mutants. The three rev genes may be analyzed within this framework. The question posed is do the three rev genes block reactions in a single linear pathway or are the blocks located in parallel pathways? In an attempt to answer this question, multiple rev mutants were constructed. The UV-survival and UV-reversion phenotypes of these were compared to the phenotypes of single rev strains.

Multiple rev strains were made by crossing two strains each carrying a different rev gene and arg4-17. After sporulation the asci were dissected. The rev genotype for each spore was determined by crossing each spore to two tester strains. Each tester strain carried one of the two rev genes in the cross, and both carried arg4-17. These test diploids were then tested for UV-induced reversion of arg4-17, carried in homozygous condition. Since different rev genes exhibit complementation for the UV-reversion function, lack of UV-revertants indicated homozygosity of rev genes in the test cross. The following multiple rev strains were identified by this method:

XY120-6C	rev1-1 rev2-1	arg4-17
XY123-7B	rev1-1 rev3-1	arg4-17
XY121-2A	rev2-1 rev3-1	arg4-17
XY124-2C	rev1-1 rev2-1 rev3-1	arg4-17

The single rev strains were the same as those discussed previously:

X1687-16C	REV	arg4-17
XY6-5A	rev1-1	arg4-17
XY36-3D	rev2-1	arg4-17
XY19-1D	rev3-1	arg4-17

The survival curves for these 8 strains are shown in Figure 15. The strain carrying both rev1-1 and rev2-1 is much more UV-sensitive than those with either rev1 or rev2 (DRF of 2). This suggests that rev1 and rev2 act in different pathways to block the repair of UV-irradiated DNA as measured by colony survival. The strain carrying both rev1 and rev3, however, appears to be no more UV-sensitive in the low dose region than the strain carrying rev3 alone. The meaning of the resistant tail in the high dose region of the UV-survival curve is not known, but probably is not due to a mixed population of cells because of the procedure used in growing the cultures. The results suggest that REV1 and REV3 act in the same linear pathway. If this linear pathway is parallel to the pathway in which REV2 acts, then one would predict that a rev2 rev3 strain might be significantly more UV-sensitive than the rev3 strain, similar in kind to the case with rev1 and rev2. This, however, is not observed. The rev2 rev3 strain is only slightly more UV-sensitive than the rev3 strain. This suggests that the REV2 pathway produces a substrate that can be utilized as an intermediate in the pathway specified by REV1 and and REV3. The following general scheme is proposed:

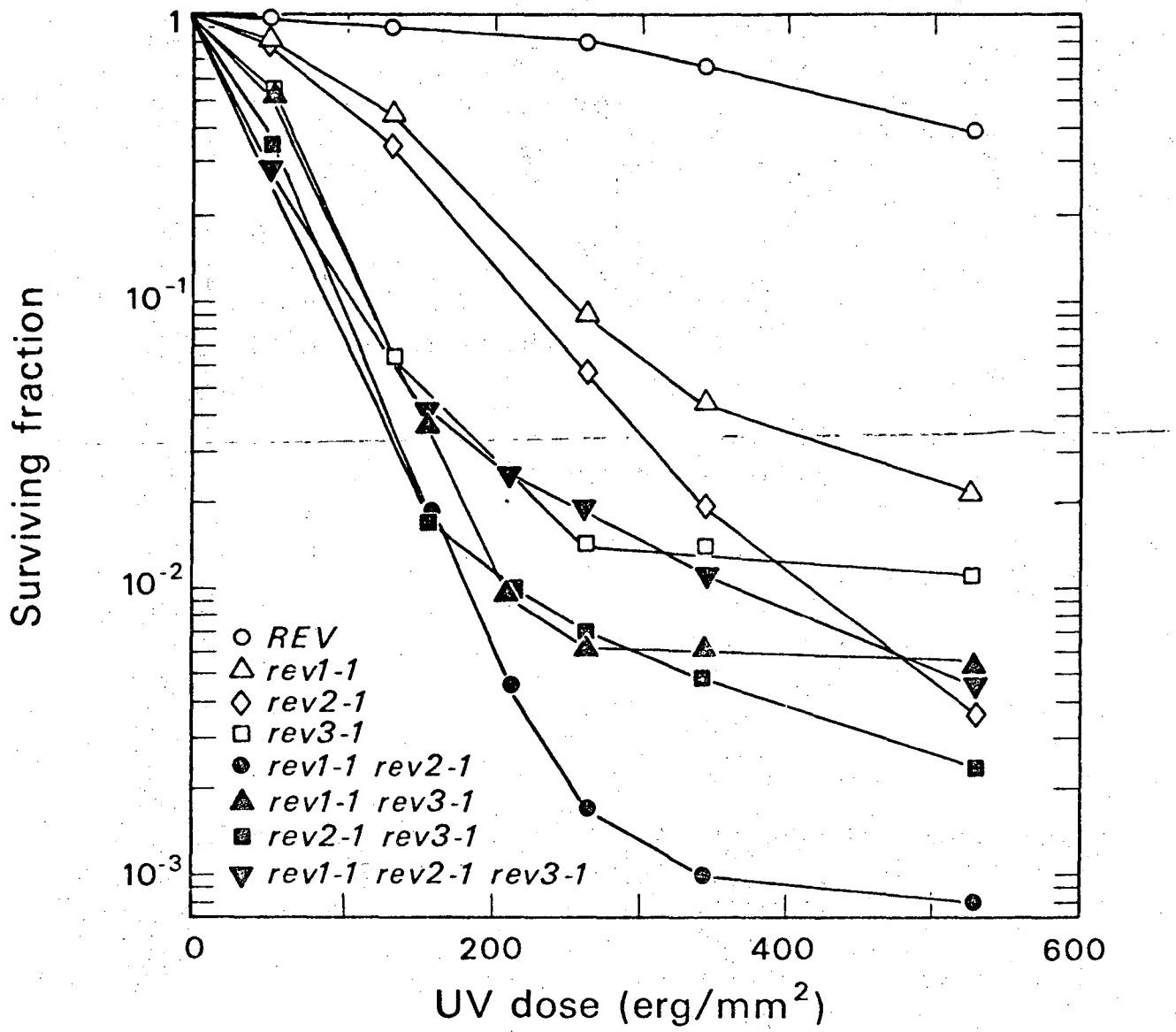

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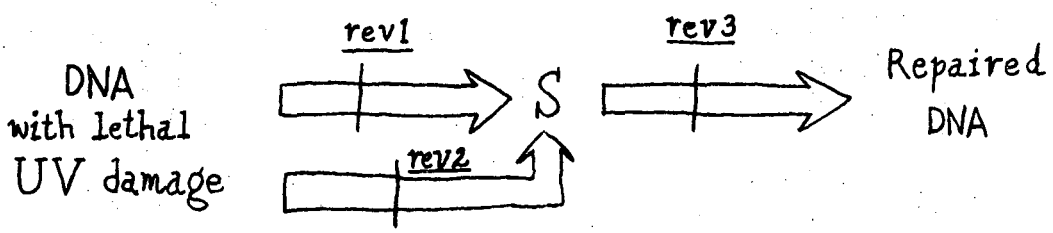
Figure 15. --UV-survival curves of haploid strains carrying zero (REV), one or more rev loci.



The letter S denotes an unknown intermediate. The large arrows denote pathways containing an unknown number of steps.

This scheme is supported by two additional experimental findings. First, the UV-sensitivity of the rev1 rev2 strain is essentially the same as that of the rev3 strain. This result is consistent with the above scheme since substrate S will not be produced by either the rev1 branch or the rev2 branch in this double mutant. This will have the same effect as a block in the formation of repaired DNA by rev3. Second, the UV-sensitivity of the triple mutant strain carrying rev1, rev2 and rev3 is essentially the same as that of the rev3 strain. This further supports the proposed scheme by suggesting that the reaction blocked by rev3 occurs later than those blocked by rev1 and rev2.

Since substrate S in the proposed scheme can be generated by either the REV1 branch or the REV2 branch, a single mutation in one branch would still allow substance S to be made by the other branch. A single mutation such as rev3 occuring after substance S, however, would block the production of repaired DNA by this pathway. Thus, rev3 mutations would be expected to have a greater effect on the repair of lethal UV damage than rev1 or rev2 mutations.

This is observed. Strains carrying rev3-1 alone are about twice as UV-sensitive as those carrying rev1-1 or rev2-1 alone.

The above scheme applies only to lethal UV damage. If premutational damage is converted into mutations by the action of REV genes in these same pathways, then all multiple rev strains would be severely blocked in UV-induced mutation like the rev3 phenotype. UV-induced locus reversion of arg4-17 was measured in multiple rev strains. All multiple rev strains exhibited locus reversion frequencies of zero in the dose range examined, i.e., less than 133 erg/mm^2. All of the viable revertants were found to carry suppressors. The rev3-1 single mutant strain is the most severely blocked in UV-reversion of all the rev strains. These results indicate that the UV-reversion phenotype of all multiple rev strains is similar to that expressed by the rev3 strain and that the scheme proposed for lethal DNA damage as initial substrate also applies to premutational DNA damage.

2. Double mutants of rev and uvs9. Both Snow (94) and Resnick (76) have isolated mutants at the uvs9 locus. Such mutants are very UV-sensitive and exhibit DRF's between 20 and 30, compared to wild-type (76). The rev mutants, however, are only moderately UV-sensitive and exhibit DRF's at 40% survival between 3.7 and 8.5, compared to wild-type. UV-survival curves are shown in Figure 16 for the following strains:

X1687-16C	a	REV
XY6-5A	a	rev1-1
XY36-3D	a	rev2-1
XY19-1D	a	rev3-1
X2947-4C	α	uvs9-2

X2947-4C is a meiotic segregant of the original uvs9-2 mutant isolated by Resnick (76). Figures 16 and 15 reveal that the DRF of this strain at 40% survival is 31, compared to the wild-type.

Resnick (79) showed that a strain carrying uvs9 is not only very UV-sensitive to colony survival, but is also very sensitive to the mutagenic effects of UV. That is, at equal UV doses, the frequency of mutation among the survivors was much greater in the uvs9 strain than in the wild-type strain. A similar UV-mutational response is seen in excision-defective (hcr) E. coli B, in which unexcised pyrimidine dimers lead to the production of mutations in the surviving cells (29, 105). At equal UV doses the hcr strain is left with many more pyrimidine dimers than the wild-type, after the time for excision has elapsed. It is possible that the UVS9 locus in yeast is one that controls excision of UV damage. Since mutations at either uvs9 or rev loci cause cells to be UV-sensitive, it is of interest to know whether any of the REV genes are involved in pathways that also involve UVS9. It would also be important to know whether rev genes can block the production of UV-induced mutations in a strain carrying uvs9, i.e., in the uvs9 rev double mutant strain. If true, this could mean that the REV gene acts to convert unexcised UV damage into mutations, assuming

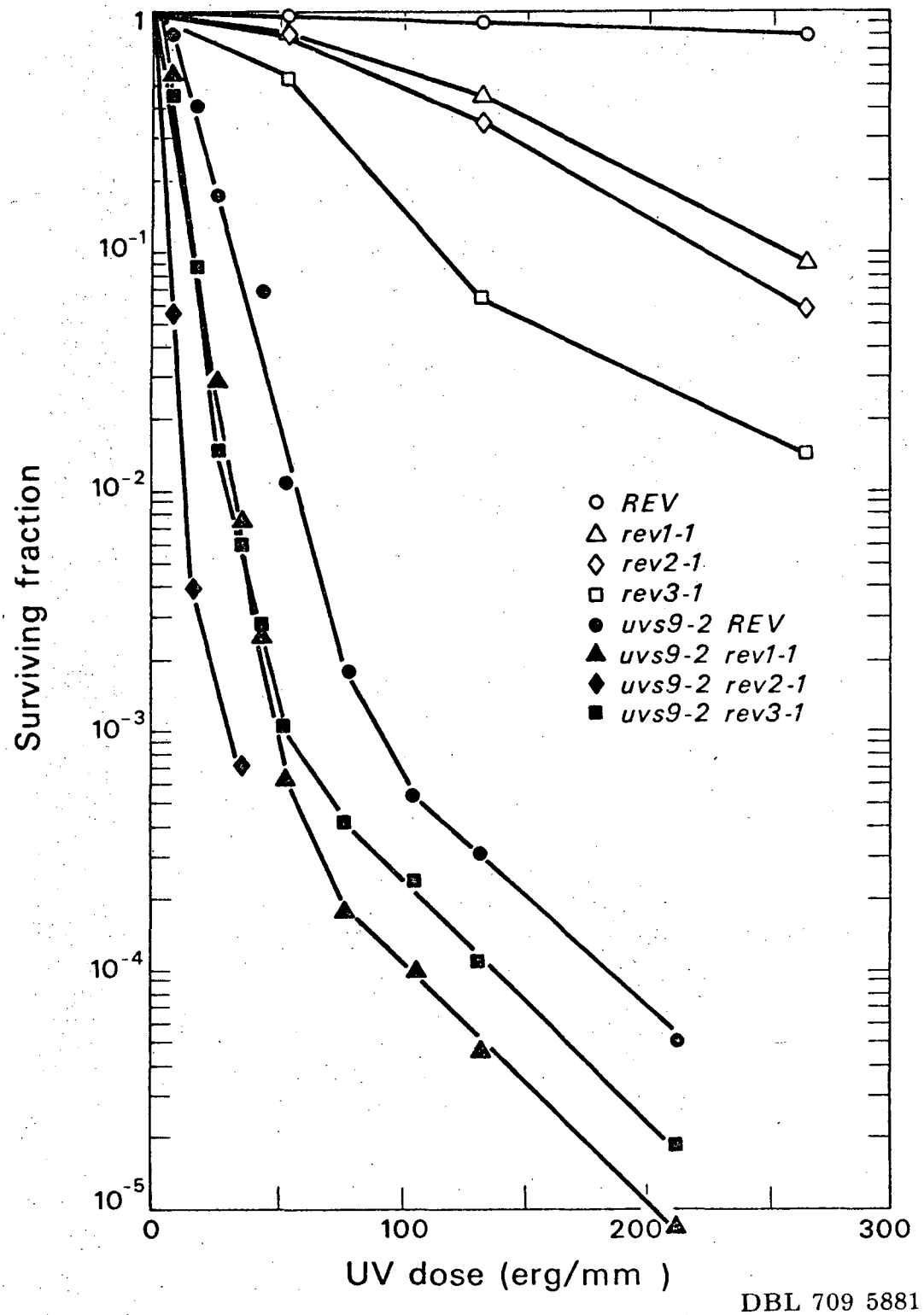


Figure 16.--UV-survival curves of uvs9-2 REV and and uvs9-2 rev double mutant haploid strains. UV-survival curves of REV and rev single mutant strains are also shown.

uvs9 blocks excision.

The study of double mutants can reveal possible relations between pathways controlled by REV genes and by the UVS9 gene. If UVS9 acts in a pathway that includes a REV gene, then a double mutant strain, uvs9 rev should not be any more UV-sensitive than the uvs9 strain. If, however, UVS9 acts in a pathway that does not include a REV gene, then this double mutant strain should be more UV-sensitive than one carrying uvs9 alone.

To construct these uvs9 rev strains, each of the rev strains described above, XY6-5A, XY36-3D, and XY19-1D was crossed to the uvs9 strain, X2947-4C. After sporulation the asci were dissected. The spore clones in each cross were replica-plated onto C-AR and the replicas tested for UV-induced reversion of arg4-17. Some of the asci consisted of two spores exhibiting a wild-type reversion phenotype and two spores with no revertants. These latter two spores were considered to carry both uvs9-2 and rev. The following strains were identified:

XY80-6A	uvs9-2	rev1-1	arg4-17
XY79-19A	uvs9-2	rev2-1	arg4-17
XY81-3D	uvs9-2	rev3-1	arg4-17

The survival curves for these three strains are shown in Figure 16. To obtain more accurate curves for these strains, which are extremely sensitive to UV, the intensity of the UV lamp was reduced by a factor of three to 8.8 erg/mm^2/sec. It is clear that these three double mutant strains are more UV-sensitive than the

uvs9 strain. The DRF's at 1% survival for strains carrying uvs9-2 rev1-1, uvs9-2 rev2-1 and uvs9-2 rev3-1 are 1.7, 4.1 and 1.9, respectively, using the uvs9-2 strain as reference. These results suggest that the three REV genes act in pathways that do not involve UVS9.

Thus, assuming that uvs9 blocks excision repair, it would seem unlikely that rev genes do also, especially in view of the different reversion responses of the uvs9 and rev single mutant strains. If rev genes blocked excision repair in pathways parallel to that of uvs9, mutations in two of these genes could, in principle, reduce the concentration of intracellular excision enzyme. The uvs9 rev double mutant strains would be more UV-sensitive than single mutant strains according to this hypothesis. If this were true, then the double mutant strain would be not only more UV-sensitive than the uvs9 strain, but also more sensitive to UV-mutation, since a greater number of unexcised dimers would exist as premutational lesions. Figure 17 shows the reversion induction curves for the locus reversion of arg4-17 versus UV dose in the three double mutant strains compared to the uvs9 strain. The curves show that these double mutant strains are less sensitive to UV-induced mutation than the uvs9 strain. The induction of revertants of arg4-17 is severely reduced in all three uvs9 rev double mutant strains. This shows that a majority of the premutational lesions in the uvs9 strain are not converted into

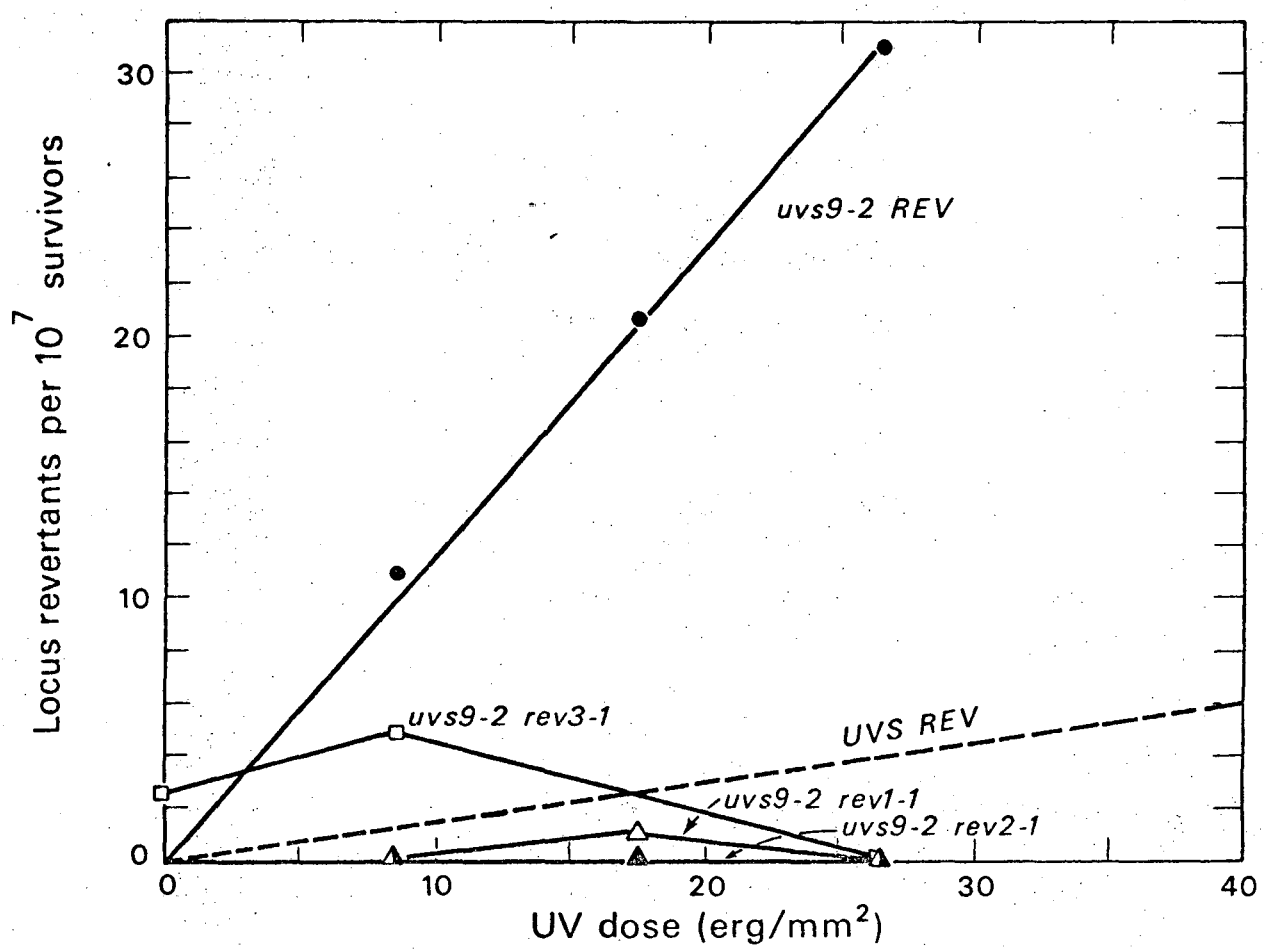


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Figure 17. --Locus reversion of arg4-17 vs. UV dose in UVS REV, uvs9-2 REV and uvs9-2 rev double mutant haploid strains.

mutations when a rev gene is also present, but rather, they remain as lethal lesions. The rev genes are epistatic to uvs9 with regard to the mutation phenotype since they suppress the phenotype of the latter.

J. Recombination in rev/rev diploids

Recent studies in E. coli have revealed that genes controlling genetic recombination (58, 108) play a role in the production of UV-induced mutations. If the mechanisms of both UV-induced mutagenesis and of recombination in yeast are similar to those in bacteria, then a priori one might expect to find that some genes involved in mutation induction also regulate recombination. The question is whether any of the rev genes isolated in this study are analogous to the rec genes in bacteria. It is reasonable then to ask whether rev genes can interfere with genetic recombination in yeast. Both mitotic and meiotic recombination were studied in diploids homozygous for rev genes. Both intergenic and intragenic systems were investigated.

1. Induced mitotic recombination

a) Intergenic recombination. Both UV-induced and X-ray induced mitotic recombination were studied in the following strains:

XY129	REV/REV	ADE/ade2-1
XY150	rev1-1/rev1-1	ade2-1/ADE
XY127	rev2-1/rev2-1	ade2-1/ADE
XY128	rev3-1/rev3-1	ADE/ade2-1

Mitotic recombinational events were scored in the region between ade2 and its centromere. The ade2 locus maps on fragment 1 and has a SDS frequency of 75.9% (61). When present in homozygous or hemizygous condition, ade2 leads to the accumulation of an intracellular red pigment which is readily seen at the clonal level (80). Thus, if a single reciprocal mitotic cross-over event should occur between ade2 and its centromere in a diploid heterozygous for ade2, then ade2 would be expected to segregate to form one daughter cell homozygous for ade2 and one daughter cell homozygous for ADE wild-type. If both daughter cells are viable, then the resultant clone would be sectored--half red and half white. If a radiation-induced division delay occurred in one of the two segregants, or if both cells experienced unequal division delays, or if the growth rates of the two segregants are different, then the red sector would appear as greater or less than half of the clone. If the ADE/ADE segregant does not form a clone, then whole red colonies would result. Non-reciprocal recombination (or gene conversion) could also occur especially at higher doses and lead to red-sectored clones (67). Nakai and Mortimer (67) found that most of the UV and X-ray induced events leading to homozygosis were reciprocal in nature.

Cells of the strains listed above were plated out onto YEPD. Some plates were exposed to varying doses of X-rays, some to UV, and some received no irradiation. After incubation for about 5 days

at 30°C, red sectors were scored. Whole and partial red colonies were added together. The frequencies of red sectors were calculated by dividing the total number of red sectors by the total number of surviving clones examined. The data are given in Table 7 and the corresponding UV and X-ray induction curves are plotted in Figures 18 and 19, respectively.

It can be seen that rev/rev diploid strains exhibit an enhanced response to induced mitotic recombination for nearly every dose of both UV and X-rays, when compared to the REV/REV diploid strain. Snow (95) and Zakharov et al. (111) have observed that diploids homozygous for other genes conferring UV-sensitivity exhibit a greater response to UV-induced intergenic mitotic recombination. The role of recombination in UV-mutagenesis is considered in Discussion.

b) Intragenic recombination. Diploid carrying homozygous rev genes were examined for the ability to interfere with both X-ray and UV-induced intragenic mitotic recombination between two arg4 alleles in repulsion, arg4-6 and arg4-17. Such recombination, also known as heteroallelic reversion, is mostly non-reciprocal (102). Prototrophs are produced by gene conversion of one allele. These alleles were estimated to be only about 2.2 X-ray map units apart (59). Co-conversions in meiosis would occur more frequently than single-site events and do not produce prototrophs (18a). If mitotic conversions are also mostly

Table 7. --Induction of mitotic recombination in the ade2-centromere region by UV and X-rays in REV/REV and rev/rev diploids.

	XY129 REV/REV				XY150 rev1-1/rev1-1				XY127 rev2-1/rev2-1				XY128 rev3-1/rev3-1			
	%S	N	Sect.	% Sect.	%S	N	Sect.	% Sect.	%S	N	Sect.	% Sect.	%S	N	Sect.	% Sect.
UV (erg/mm^2)																
0	100	963	3	0.3	100	1423	1	0.07	100	1738	40	2.3	100	1340	0	0.07
265	100	316	6	1.9	77	610	35	5.7	66	507	28	5.5	100	262	19	7.2
530	96	1081	23	2.1	72	1020	96	9.4	5.8	467	39	8.3	4.7	465	19	4.1
795	63	915	28	3.1	1.2	696	60	8.6	0.75	631	44	7.0	0.74	376	13	3.5
X-ray (Kr)																
0	100	963	3	0.3	100	1423	1	0.07	100	1738	40	2.3	100	1340	0	0.07
3.75	97	297	3	1.0	98	317	7	2.2	92	462	8	1.7	100	388	9	2.3
7.50	94	460	9	2.0	85	563	25	4.4	86	482	23	4.8	91	326	13	4.0
15.0	85	947	32	3.4	49	437	22	5.0	64	622	17	2.7	87	473	31	6.6
45.0	22	334	21	6.3	0.13	165	12	7.2	7.5	98	20	20.2	4.0	25	7	28.0

Note: %S = percent survival, N = total number of surviving clones, Sect. = total number of red sectors (includes whole and partial red colonies).

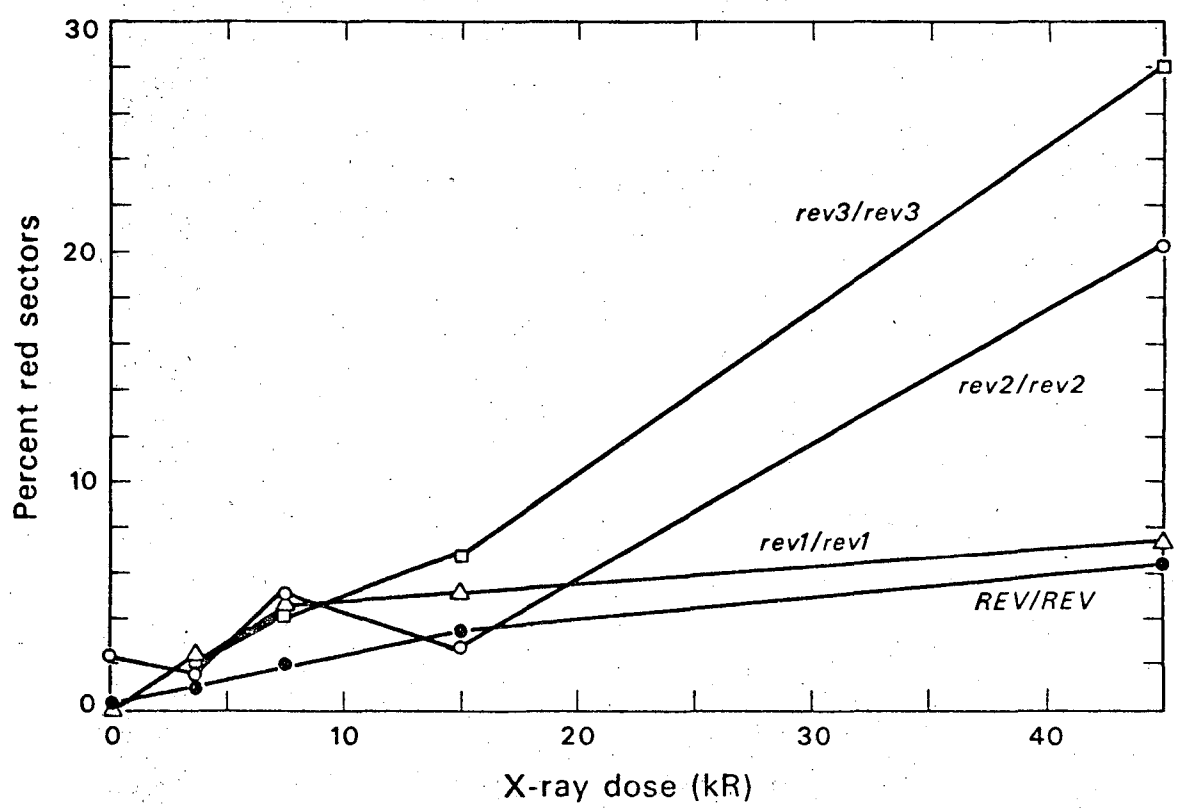

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Figure 18.--X-ray induced intergenic mitotic recombination between ade2 and its centromere in REV/REV and rev/rev diploid strains.

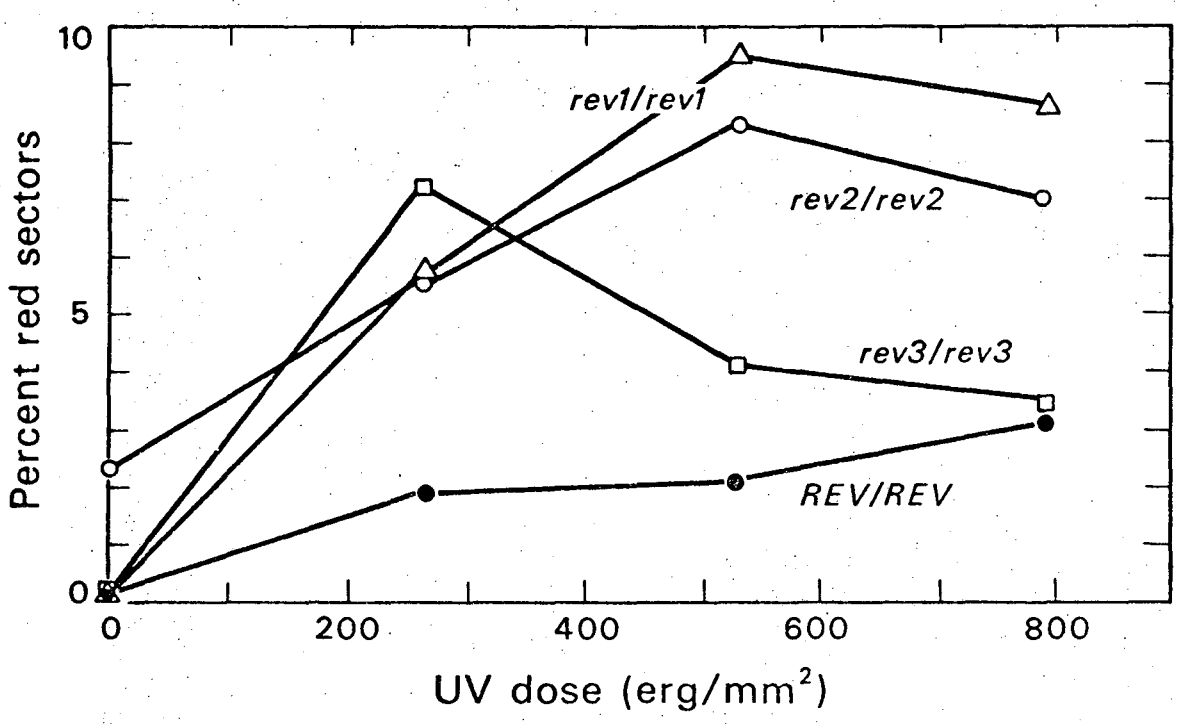

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Figure 19. --UV-induced intergenic mitotic recombination between ade2 and its centromere in REV/REV and rev/rev diploid strains.

co-conversions of these alleles, then the observed prototrophs represent a minority population of single-site conversions among all conversions.

Most rev haploid strains routinely carry the arg4-17 allele. These strains were crossed to strains carrying arg4-6. After sporulation haploid spores carrying both rev and arg4-6 were identified according to the procedure described previously. A rev arg4-6 segregant was then crossed to a rev arg4-17 segregant to form the following heteroallelic diploids:

XY 182	REV/REV	arg4-6/arg4-17
XY 183	rev1-1/rev1-1	arg4-6/arg4-17
XY 184	rev2-1/rev2-1	arg4-6/arg4-17
XY 185	rev3-1/rev3-1	arg4-6/arg4-17

Cells of these strains were cultured in liquid YEPD and harvested according to standard procedures described in Materials and methods. Large numbers of cells ($\sim 10^6$) were plated onto C-AR plates and subsequently exposed to varying doses of UV or X-rays to induce mitotic recombinants. The doses employed were small in that cell viability remained at about 100% after all exposures to both inducing agents. This was verified by plating appropriate dilutions of cells onto YEPD and measuring the survival at each dose. The UV and X-ray induction curves are given in Figures 20 and 21, respectively. The data points for the plot of X-ray induced prototrophs per 10^5 survivors versus X-ray dose were fitted to a straight line by the least squares method, since such a measurement is known to

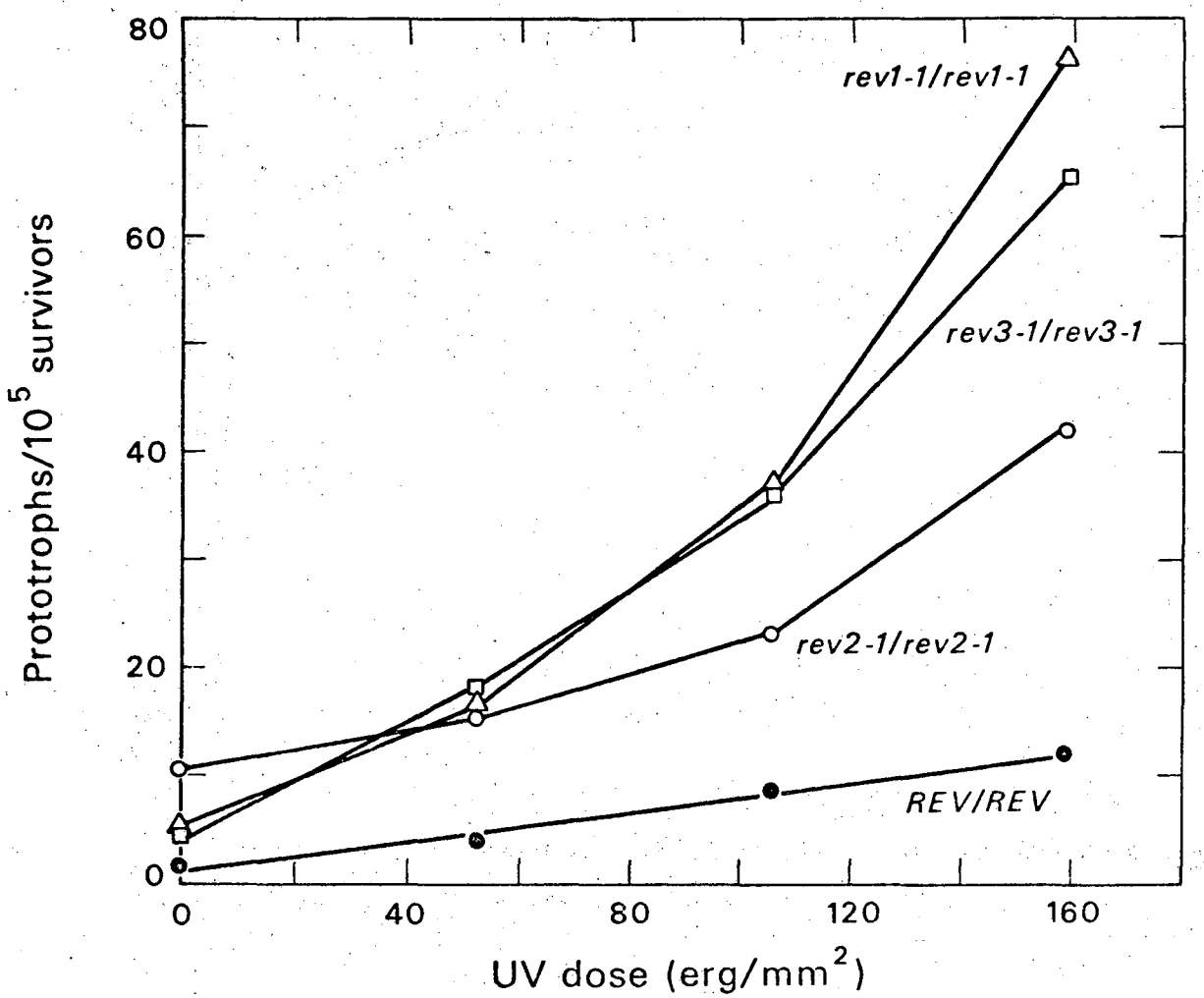

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Figure 20.--UV-induced intragenic recombination between arg4-6 and arg4-17 in REV/REV and rev/rev diploid strains.

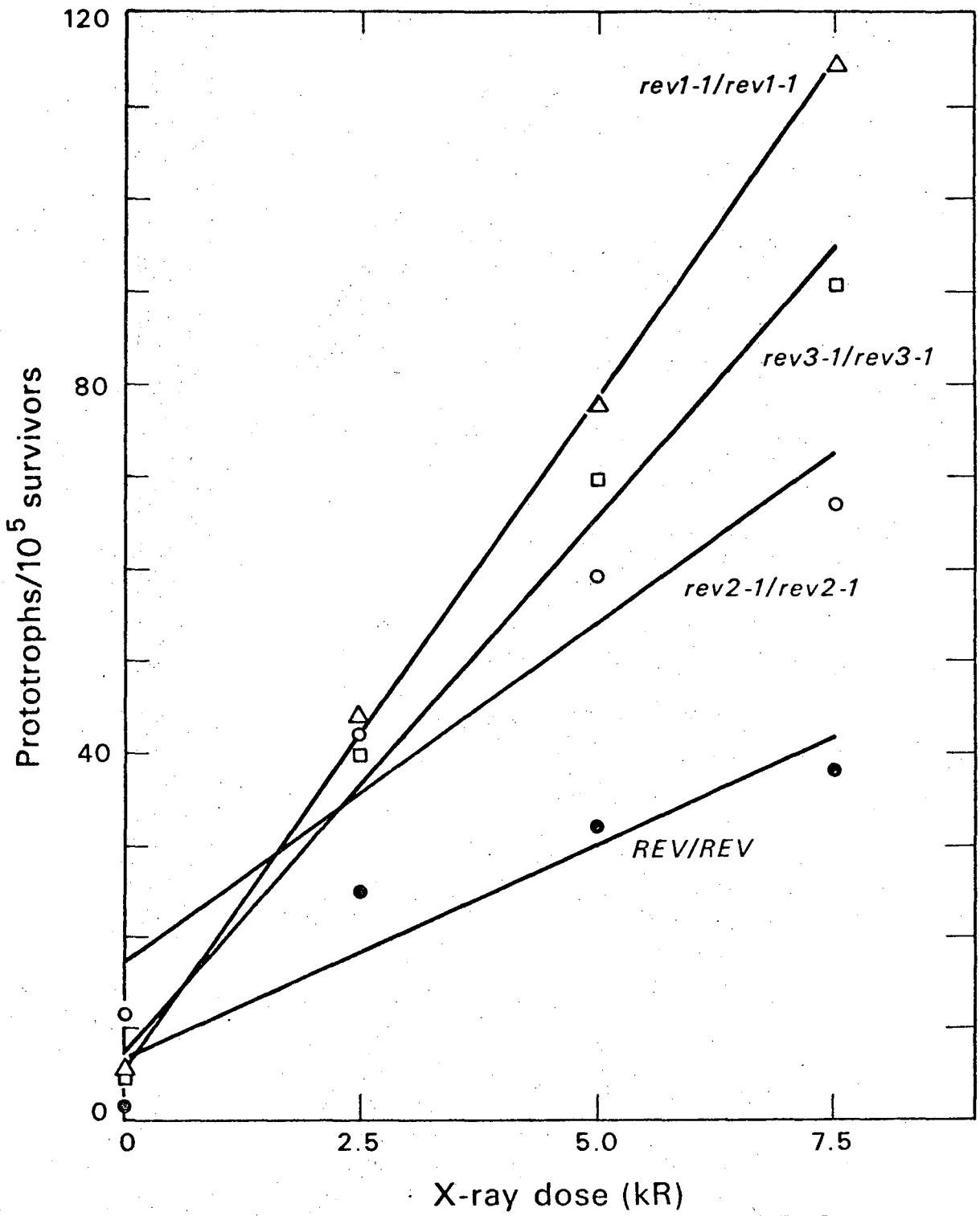

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Fig. 21.--X-ray induced intragenic recombination between arg4-6 and arg4-17 in REV/REV and rev/rev diploid strains.

increase linearly with X-ray dose (55).

Figure 21 shows that homozygous rev diploids exhibit an enhanced response to X-ray induced heteroallelic mitotic recombination, compared to the wild-type response as measured by this system. The slopes and respective standard errors of the regression lines for XY182, XY183, XY184 and XY185 are 4.7 ± 1.2, 14.5 ± 0.3, 7.3 ± 1.5, and 11.5 ± 0.9 prototrophs per 10^8 survivors per roengten, respectively. Figure 20 shows that the rev/rev diploids also exhibit an increased response to UV-induced intragenic mitotic recombination. This has also been observed by Snow (95) in diploids homozygous for other genes controlling UV-sensitivity. As with X-rays the degree of enhancement of recombination by UV in rev1-1/rev1-1 and rev3-1/rev3-1 strains is greater than in the rev2-1/rev2-1 strain, compared to wild-type. If, with increasing proximity of the two heteroalleles, co-conversions are produced mitotically at the expense of single-site conversions (18a), the enhanced recombination of rev/rev diploids could be due to an increased proportion of single-site events. This could occur if rev genes reduce the length of the converted interval.

The frequencies of UV-induced locus reversion of arg4-17 are considerably more reduced in rev1-1/rev1-1 and rev3-1/rev3-1 diploids than they are in the rev2-1/rev2-1 diploid, compared to wild-type. A similar effect was observed with respect to locus reversion of lys1-1. Thus, rev2-1 has the least effect, compared to the

rev1-1 and rev3-1 alleles, in both reducing UV-reversion and enhancing induced mitotic recombination, relative to REV. This correlation suggests the following: REV pathways may produce UV-mutations by utilizing a substrate that also stimulates recombination by a different mechanism. If rev2 blocks a minor parallel branch of these mutational pathways, a small amount of additional substrate can stimulate recombination. If the rev1 or rev3 branches are blocked, however, a large amount of additional substrate can stimulate recombination. The role of recombination in UV-mutagenesis is considered in Discussion.

2. Meiotic recombination.

a) Intergenic recombination. Diploid strains carrying homozygous rev genes were examined for their ability to interfere with meiotic recombination between the two linked genes leu1 and trp5. Both genes map on the same arm of chromosome VII and are separated by a distance of 13.3 cM (61). The following strains were used:

XY129	REV/REV TRP/trp5-48	arg4-17/arg4-17 ADE/ade2-1	LEU/leu1-12
XY150	rev1-1/rev1-1 TRP/trp5-48	arg4-17/arg4-17 ADE/ade/2-1	LEU/leu1-12
XY127	rev2-1/rev2-1 TRP/trp5-48	arg4-17/arg4-17 ADE/ade2-1	LEU/leu1-12
XY128	rev3-1/rev3-1 TRP/trp5-48	arg4-17/arg4-17 ADE/ade2-1	LEU/leu1-12

These are the same strains as those used to study intergenic mitotic recombination. These strains have already been shown to be UV and

X-ray sensitive. The diploids were sporulated, treated with glusulase enzyme and sonicated as described in Materials and Methods. The random spores were then plated out onto YEPD and incubated at 30°C to allow clones to develop. Clones were then tested for adenine, leucine, and tryptophan deficiencies on C-AD, C-LE, and C-TR, respectively. Only adenine-deficient clones were examined for leucine and tryptophan requirements to avoid including unsporulated diploids in the random spore sample. The ade2 gene segregates independently of leu1 and trp5(61). Each clone was classified by this method as having either parental or recombinant phenotype with respect to the leucine and tryptophan markers. The data are shown in Table 8. It can be seen that the percentage recombinant spores for the three rev diploids is not significantly different from that of the wild-type. These data indicate that intergenic meiotic recombination in the leu1 - trp5 region is not significantly different from the wild-type frequency when the diploid strain is homozygous for rev genes.

b) Intragenic recombination. Diploids carrying homozygous rev genes were examined for the ability to interfere with meiotic recombination between arg4-6 and arg4-17. The same heteroallelic diploids were studied as in intragenic mitotic recombination.

XY182	REV/REV	arg4-6/arg4-17
XY183	rev1-1/rev1-1	arg4-6/arg4-17
XY184	rev2-1/rev2-1	arg4-6/arg4-17
XY185	rev3-1/rev3-1	arg4-6/arg4-17

Table 8.--Intergenic meiotic recombination between leu1 and trp5 in REV/REV and rev/rev diploids.

Diploid	*rev* Genotype	Total no. AD^- ascosporal clones	Parental LE^+TR^+	Parental LE^-TR^-	Recombinant LE^-TR^+	Recombinant LE^+TR^-	% Recombinant	$\chi^2 \times 10^2$	$P(\chi^2)$
XY129	*REV/REV*	113	52	41	12	8	17.7		
XY150	*rev1-1/rev1-1*	132	68	40	8	15	17.4	0.075	>.98
XY127	*rev2-1/rev2-1*	108	54	37	9	8	15.7	14.7	.70
XY128	*rev3-1/rev3-1*	100	47	35	10	8	18.0	0.52	>.90

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* Expected values calculated in each case from the mean response of REV/REV and rev/rev strains.

The same single colony isolate was used for both the mitotic and the meiotic recombination measurements. Meiotic recombination between the two alleles was studied by means of random spore analysis. The diploids were sporulated and the asci treated with glusulase. After sonication, the random spore cultures, containing unsporulated diploids as well, were plated onto C-AR plates. Six plates were used for each culture. Between 3000 and 9000 cells (haploids and diploids) were spread onto the C-AR plates, as measured by the plating of an appropriate dilution on YEPD to obtain the viable titre. After incubation, the arg+ revertant colonies (prototrophs) were counted. The prototrophs were also replica-plated to other omission media to verify that they did not contain suppressor mutations of arg4-17.

The amount of contamination of the random spore sample with unsporulated diploids was estimated by the following procedure: Cells from the random spore sample were diluted and plated onto YEPD to yield about 50 colonies per plate. After incubation, plates containing these clones were replica-plated onto C-AR plates. These were subsequently exposed to an X-ray dose of 10 Kr and then incubated for about 3-4 days. Diploid clones could be identified by the presence of numerous X-ray induced prototrophs growing on the C-AR replicas. This was possible since rev/rev diploids are not deficient in such recombination (Figure 20). Haploids, however, containing either the arg4-17 or the arg4-6 allele, or rarely both,

did not exhibit any arg+ prototrophs, since the frequency of X-ray induced reversion of either allele is several orders of magnitude lower than the frequency of heteroallelic mitotic recombination. By this method the number of diploids was estimated for each culture from which the percentage of haploid cells among the total number of cells in the random spore sample was determined.

The frequencies of arg+ prototrophs (per haploid cell) are given in Table 9 for the various rev/rev diploids. These frequencies of meiotic intragenic recombination are not significantly different from that in the wild-type. The data presented here and in the previous section indicate that rev genes do not affect either intragenic or intergenic meiotic recombination in the systems studied. Snow (95) found that other UV-sensitive genes also had no effect on either type of meiotic recombination. These results suggest that both UV-mutagenesis and the repair of lethal UV damage are carried out in pathways that do not involve the steps leading to meiotic recombination.

Table 9.--Intragenic meiotic recombination between arg4-6 and arg4-17 in REV/REV and rev/rev diploids.

Strain	*rev* Genotype	Viable cells plate $\times 10^{-2}$	% Haploid ascosporal clones	Viable haploid cells plate $\times 10^{-2}$	Average no. prototrophs /plate	Freq. of prototrophs per viable haploid spore $\times 10^{-2}$
XY182	*REV/REV*	38.4	53.2	20.4	19±3.6	0.93±0.18
XY183	*rev1-1/rev1-1*	56.7	31.0	17.6	22.7±3.3	1.29±0.19
XY184	*rev2-1/rev2-1*	30.0	53.0	15.9	16.8±2.8	1.06±0.18
XY185	*rev3-1/rev3-1*	82.5	47.0	38.8	48.8±4.4	1.26±0.11

DBL 708 5856

* Denotes standard error of six plate counts.

IV.

DISCUSSION

A. The control of mutation induction by rev genes.

The present study has concerned itself with the genetic control of mutation induction in yeast, in particular UV-induced mutation. To determine whether there are any similarities between UV-mutagenesis in yeast and in bacteria, mutants were selected for reduced UV-mutational response. The phenotypes of such mutants were investigated with the hope of answering questions concerning the number of genes controlling UV-induced mutation, the specificity of such genes, the relation between mutation induction and the repair of lethal DNA damage, and the role of recombination in mutagenesis.

The twenty reversionless mutants identified represent only three unlinked recessive genes. Strains carrying these genes are moderately UV-sensitive. In contrast, twelve UV-sensitive mutants exhibiting reversion ability were found to represent 11 complementation groups. Cox and Parry (14) found 22 loci that controlled UV-sensitivity in yeast. Thus, it would appear that most UV-sensitive mutations leave the UV-induced mutation phenotype unchanged or

enhanced, while very few result in a reduced UV-mutational response. Assuming that REV gene products are enzymes involved in UV-mutagenesis, the unusually small number of rev loci identified suggests that UV-induced mutation in yeast involves a small number of enzymatic reactions.

The three rev genes do not appear to be locus specific in that their action of reducing UV-reversion is not confined to the reversion of only arg4 alleles. These genes can also reduce UV-induced locus reversion of the lys1-1 allele. As in the case of arg4-17 locus reversion, rev2-1 does not reduce lys1-1 locus reversion as much as either rev1-1 or rev3-1 can.

In the REV strain the frequencies of locus reversion of lys1-1 are less than those of arg4-17. Presumably, both arg4-17 and lys1-1 code for the same nonsense codon UAA in messenger RNA (20, 26, 28). The mutant triplets in DNA are the same in both cases--TTA in one strand and TAA in the other. There is a 50% chance that the base immediately adjacent to the thymine on the end of each triplet in either strand is a pyrimidine in both cases. Thus, both alleles contain at least two adjacent pyrimidines that can dimerize after UV exposure and cause a base-pair substitution mutation. The nature of the bases on either side of the triplets might account for the differences in reversion induction frequencies. In addition, the possible amino acid replacements acceptable to each site may be different. Sherman (88) found different

distributions of amino acid replacements among revertants of two nonsense alleles, located at different positions in the iso-1 cytochrome c cistron. The molecular environment of an allele evidently plays an important role in its reversion pattern.

The possibility that rev genes could be allele-specific in reducing reversion of only ochre-suppressible alleles was tested by studying UV-reversion of arg4-6, likely a missense allele (59), in strains carrying both arg4-6 and rev. Both rev1-1 and rev3-1 significantly reduce UV-reversion of arg4-6, while rev2-1 has no effect or a possibly slightly negative effect in reducing reversion. This suggests that rev1-1 and rev3-1 have a non-specific action in reducing UV-reversion of both nonsense and missense alleles, while rev2-1 may be specific for nonsense alleles. Base-pair substitutions can result in reversion of both kinds of alleles (76). No evidence was obtained in the present study to suggest a mechanism by which rev2-1 reduces reversion of only nonsense alleles.

B. Mutation induction and the repair of lethal DNA damage

Cells carrying rev genes isolated in this study are sensitive to the inhibition of colony-formation after exposure to agents known to induce damage in DNA. Cells carrying any one of the rev genes are moderately UV-sensitive, slightly X-ray sensitive, and EMS-sensitive. There is considerable evidence (81, 82, 99) that mutants of E. coli sensitive to these agents are unable to carry out certain

steps in the repair of lethal DNA damage induced by these agents. Radiation-sensitive mutants of yeast are probably also defective in such repair processes (76, 94). Since UV is known to be mutagenic to cells and since rev genes reduce UV-mutation induction as well as confer UV-sensitivity, this suggests that UV-induced mutations in yeast are produced by pathways that share some common steps with the pathways for repair of lethal UV damage. Since rev genes also confer X-ray and EMS-sensitivity, it is likely that the repair pathways for lethal X-ray and EMS damage share some common steps with the pathways of UV-induced mutation. This will be discussed later.

The uvs9 locus in yeast causes extreme sensitivity to both the lethal (94) and mutagenic (79) effects of UV. That is, at equal UV doses the frequencies of mutation are greater in uvs9 strains than in UVS strains. At the same time, the surviving fraction is considerably lower in uvs9 strains compared to UVS strains. This stands in contrast to the fact that in rev strains, although they are UV-sensitive, fewer mutations are produced among the survivors than in REV strains at equal doses. The rev uvs9 double mutant strains are all significantly more UV-sensitive than the strain carrying uvs9 alone. These double mutant strains are less sensitive to UV-induced mutation than the uvs9 strain, as measured by the induction curves of arg4-17 locus revertants. These results suggest that rev genes act in pathways separate from the uvs9 pathway. More specifically,

uvs9 is probably blocked in the repair of lethal UV damage in a pathway that does not itself produce mutations, but whose end-product may be a substrate for REV pathways which do result in UV-induced mutations.

C. UV-induced mutation in yeast

1. The repair of primary vs. secondary UV damage. The results obtained in this study are consistent with a model for UV-induced mutation in bacteria proposed by Witkin (107). Such a model is based on the notion that primary UV damage in DNA does not block DNA replication but rather causes the production of secondary damage in the newly synthesized daughter strands in the first post-irradiation DNA replication (8, 83). It is assumed that the rev1, rev2, rev3 and uvs9 strains used in this study are not leaky and that the corresponding wild-type alleles at these loci lead to enzymes involved in DNA repair. A schematic illustration of the model proposed in yeast is shown in Figure 22. The large arrows indicate that at least one linear pathway exists for each process involving an unknown number of steps, and includes the possibility that one or more other parallel pathways may also exist.

The major feature of this model requires that two independent repair processes act in sequence. The first process concerns the repair of primary or mutagen-induced lesions in both strands of DNA.

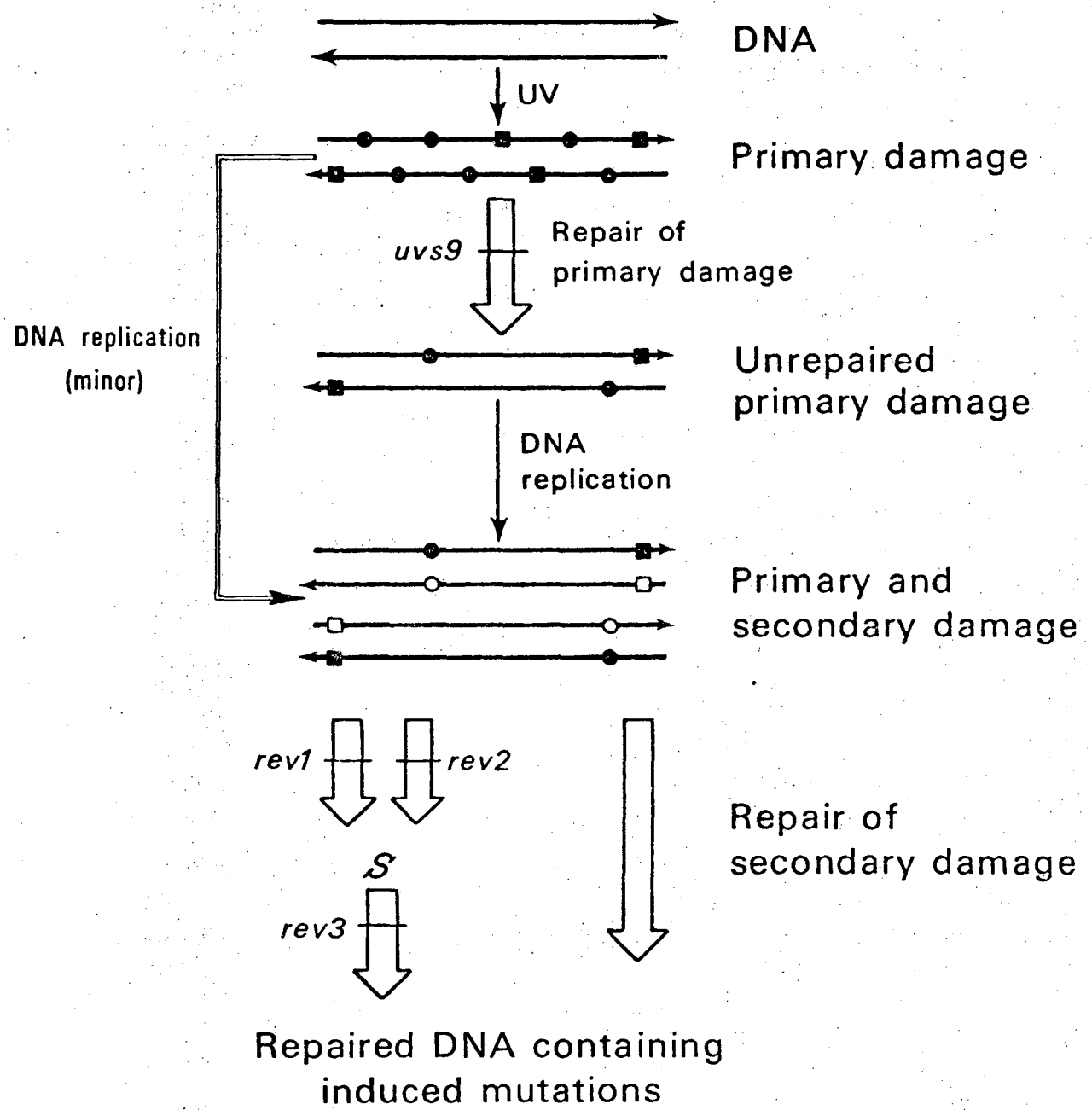

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Figure 22. Proposed model for UV-induced mutation in yeast.

Such a process acts to reduce the number of primary lesions in the parental strands before DNA replication begins. After DNA replication is completed, the unrepaired primary lesions remain in the parental strands. The daughter strands, however, contain defects opposite the primary lesions in the parental strands, perhaps because of the altered base-pairing properties of primary lesions. These defects are called secondary lesions in this model, since they were induced not by the mutagen, but by means of DNA replication on a damaged template. As seen in the model, these secondary lesions may be produced directly from primary lesions by means of DNA replication. That is, repair of primary damage need not occur before secondary lesions can be produced. Strains completely blocked in the repair of primary damage would produce secondary damage directly from primary damage by means of this minor pathway. In these strains, since virtually all primary lesions are unrepaired, the number of secondary lesions is much greater at equal UV doses than in strains able to undergo repair of primary damage.

The second process is the repair of secondary damage. It is independent of the first repair process in that secondary lesions constitute its major substrate. The amount of this substrate can vary depending on whether or not primary repair is operating. As in the Witkin model (107), it is proposed that secondary lesions, and not primary lesions, constitute the substance of the premutational

damage induced in cells by UV and that the repair of secondary lesions is the process that converts secondary damage into mutations.

The UVS9 gene has been assigned a role in the repair of primary damage, whereas the REV genes have been assigned roles in the repair of secondary damage. The evidence for these assignments has already been discussed. The data obtained in this study do not require that DNA replication is the cause of secondary damage in yeast. The evidences obtained here, however, are consistent with the interpretation proposed in E. coli.

The phenotype of uvs9 can be understood within the proposed model. At a given UV dose, an equal number of primary lesions are induced in the DNA's of both a uvs9 REV strain and the wild-type UVS REV strain. The repair of a majority of these lesions occurs in the UVS strain by the first repair process, but in the uvs9 strain, such repair is blocked or partially blocked. There may exist other parallel pathways of repair of primary damage that do not depend upon UVS9. This seems likely in view of the large number of genes controlling UV-sensitivity in general (14). Undoubtedly, many of the 22 loci studied by Cox and Parry (14) are involved in repair of primary damage. After DNA replication there are a much greater number of secondary lesions in the uvs9 strain than in the UVS strain. Since both strains effect repair of secondary damage and produce mutations from this damage, the uvs9 strain exhibits greater mutation

induction frequencies than the wild-type, and is also much more UV-sensitive than the wild-type.

The phenotypes of rev genes can also be understood using this model. At a given UV dose an equal number of primary lesions are induced in the DNA's of both a rev strain and the wild-type REV strain. Since both strains repair this damage, an equal number of secondary lesions are produced after DNA replication in both strains. The REV strain repairs this secondary damage by a mechanism that produces induced mutations among the survivors, but the rev strain is partially defective in this repair. There may be other parallel pathways of repair of secondary damage that do not depend upon REV genes. This seems unlikely in view of the finding in this study that very few UV-sensitive genes (rev genes) reduce UV-mutability. According to the model the rev strain is therefore more UV-sensitive than the wild-type. It also produces fewer mutations, since some mutational pathways have been blocked.

The phenotypes of uvs9 rev strains are consistent with this interpretation. At a given UV dose such strains produce an equal amount of secondary damage as in the uvs9 strain, but are more UV-sensitive than the uvs9 strain because they are partially defective in the repair of secondary damage. If uvs9 rev strains were not defective in this repair, they would produce mutations at the frequencies observed in uvs9 strains. If uvs9 rev strains were defective only in the repair of primary damage by a parallel pathway not including

uvs9, then they would produce mutations at frequencies significantly greater than those observed in uvs9 strains. Induced locus reversion of arg4-17 is significantly lower in uvs9 rev strains than in the uvs9 strain. These results support the idea that REV genes act in pathways of repair of secondary damage as described by this model.

2. The repair of secondary damage. The model described above assigns rev genes to blocked pathways of repair of secondary damage. Analysis of the UV-survival curves of multiple rev strains has led to the conclusion that all three REV genes do not act in a single linear pathway. Rather, REV1 and REV3 act in a single linear pathway and REV2 in another pathway parallel to the first. The data suggest a scheme in which the REV2 pathway produces some substrate (denoted by S in the model) that can be utilized by the pathway defined by REV1 and REV3. The data also suggest that the reaction blocked by rev3 occurs after the production of substrate S. This scheme has been incorporated into the model as shown in Figure 22. The nature of the substrate S remains unknown at this time. The REV2 branch seems to be a minor one for the production of UV mutations, but not so for the repair of lethal damage.

3. The nature of primary and secondary lesions. Mutants of E. coli K-12 at the uvr loci and mutants of E. coli B at the hcr loci have been shown to be blocked in excision of UV-induced pyrimidine dimers in DNA (6, 87). In view of the finding that DNA polymerase exhibits exonuclease activity and can excise thymine dimers induced

by UV (41), it seems likely that this enzyme, along with an endonuclease specific for damaged DNA (40, 98) and a ligase (21, 69), can perform excision repair. Defective excision repair in uvr or hcr strains makes these strains about 20 times more UV-sensitive than the wild-type (106), and results in UV-induced mutation frequencies very much higher than in the wild type uvr+ or hcr+ strains (29, 105). It is believed that errors in the excision repair process probably occur less frequently than 10^{-6} per excised dimer (107), and that these errors do not represent the origin of induced mutations in cells exposed to UV. The phenotypes of the UVS9 strains in yeast are very similar to the phenotypes expressed by the uvr and hcr strains in E. coli. The uvs9 strains in yeast may be deficient in excision repair of primary lesions. These primary lesions are likely to be predominantly pyrimidine dimers (76).

In E. coli K-12 mutations at any one of four loci affects ultraviolet mutability (107). UV-induced mutations in strains carrying either lex (or exr in E. coli B) or recA are completely eliminated, while recB and recC loci only reduce UV-mutability compared to wild-type. Such loci also confer varying degrees of UV and X-ray sensitivity (32) and are thought to be involved in a post-replication repair mechanism that depends upon genetic recombination (33, 107). The phenotypes of rev genes are similar to those expressed by exr and rec genes in E. coli. Assuming that uvs9 does block a step in excision repair in yeast, then REV genes may be

involved in a post-replication repair mechanism utilizing secondary damage as a substrate. This secondary damage could be single-strand breaks in daughter DNA strands opposite pyrimidine dimers. There is no direct evidence for this in yeast. The question as to whether such a repair mechanism in yeast might depend upon genetic recombination will be considered below.

4. The role of recombination in UV-mutagenesis. In *E. coli* recombination seems to play an important role in post-replication repair (33, 107). Witkin (107) has proposed that most UV-induced mutations are caused by errors introduced into the DNA during post-replication recombinational repair of daughter strand gaps. The *exr+* (or *lex+*) gene product is thought to allow errors to be produced during this repair. These errors are produced by some mechanism that also promotes UV survival, while the ability to perform any kind of recombinational repair, whether accurate or inaccurate, is thought to require the *recA+* gene product (107). Strains carrying *recA* have no recombination ability (13). The *recB* and *recC* strains have reduced recombination ability (34, 16) and *exr* strains exhibit only slightly reduced recombination (about 3 or 4-fold reduced), commensurate with their reduction in UV resistance, when compared to *rec+* strains (107).

It is reasonable therefore to ask whether the control of UV-induced mutation in yeast by *rev* genes depends upon recombination ability. The data obtained in this study indicate that none of the *rev*

genes isolated are deficient in either meiotic or UV and X-ray induced mitotic recombination, as measured by the intergenic and intragenic systems discussed in Results. One interpretation is that the production of induced mutations by REV pathways does not require recombination events of the types studied herein. In this regard the similarity of rev mutants in yeast to exr mutants in E. coli seems to break down. If some type of recombination did play a role in the production of induced mutations, then rev strains might be expected to exhibit a reduced response for that type of recombination compared to the REV strain. All three rev/rev diploids were found to exhibit an increased response to UV and X-ray induced mitotic recombination when compared to wild-type. Meiotic recombination was unaffected by homozygous rev mutations. Other UV-sensitive genes in yeast, including uvs9, however, also enhance induced mitotic recombination in homozygous diploids, while meiotic recombination is unaffected (95, 111, 64). This suggests that molecular steps needed in meiotic recombination do not play a role in UV-mutagenesis in yeast. Mitotic recombination may not be required, either, but nevertheless is stimulated by agents that also induce lethal DNA damage. Mutants that are sensitive to these agents might be unable to reduce this damage by repair and therefore would be expected to exhibit a greater induced mitotic recombination response than the wild-type which effects repair. A similar conclusion was reached by Baker (3) who found that the UV enhancement of λ bacteriophage recombination was

even greater in excision-defective (uvr) host bacteria than in wild-type hosts. In this study by Baker UV-induced structural defects in DNA were thought to lead to an increased efficiency of pairing among chromosomes, precedent to recombination by breakage and reunion. No evidence was found to imply that UV-induced recombination is directly associated with any mode of DNA repair." Such a mechanism could operate in yeast.

D. Mutations induced by mutagens other than UV

In constructing an overall hypothesis for mutation induction in yeast, it wouldbe important to know whether there are any steps in the proposed model for UV-induced mutation that also participate in the production of mutations from DNA damage induced by mutagens other than UV. That rev strains are EMS-sensitive in varying degrees as well as X-ray and UV-sensitive, suggests that there may be some common steps in the repair of lethal DNA damage induced by all three mutagens. The relationship between mutation induction and the repair of lethal DNA damage has already been discussed. Thus, the cross-sensitivity of rev strains to UV and EMS indicates that steps involved in generating UV-mutations may also act on intermediates in the repair of lethal EMS damage. The repair of EMS-induced DNA damage might occur both by pathways that generate mutations and also by pathways that do not themselves generate induced mutations. Assuming that this is true, it would be important to know whether the mutagenic steps in the repair of UV damage are

also mutagenic in the repair of EMS damage. If so, then rev strains should exhibit lower EMS-induced mutation frequencies than REV strains. The assumption that rev genes do block UV-mutagenic steps is implicit in the above hypothesis.

The rev strains exhibit significantly greater frequencies of EMS-induced forward mutation to ade1 or ade2 than wild-type, but the average frequency of UV-induced forward mutation in all rev strains was lower, similar to the results for locus reversion of both arg4-17 and lys1-1. These results suggest that while REV pathways may repair lethal EMS damage, these pathways are not involved in the production of EMS-induced mutations. The rev genes may have a general effect in reducing both forward and reverse mutation. More experiments are needed, especially to determine whether rev2-1 is specific for UV-reversion of nonsense alleles.

Zimmermann(112) tested 22 UV-sensitive mutants isolated by Cox and Parry (14) for possible cross-sensitivity to the monofunctional alkylating agent methylmethanesulfonate (MMS). Eighteen of these twenty mutants including uvs9 showed cross sensitivity to MMS. EMS-induced mutation was not studied in these mutants. In E. coli Bohme and Geissler (5) found that the hcr mutation, which controls excision repair, does not affect the sensitivity of cells to treatment with EMS. Their results clearly demonstrate that strains carrying rec mutations, namely rec-34, rec-36 and rec-38 (100) are more EMS-sensitive than rec+ strains. Assuming that these rec

mutants are defective in post-replication repair, these results indicate that EMS damage in E. coli can be repaired by recombinational repair but not by excision repair. In yeast, however, the results of Zimmermann (112) indicate that uvs9 is involved in the repair of lethal alkylation damage. The uvs9 strain was not tested for the production of mutations from alkylation damage. The results of such an experiment might determine whether this step in the repair of alkylation damage is a mutagenic one. If it is true that uvs9 controls excision, reduced frequencies of EMS or MMS-induced mutation in uvs9 strains could mean that induced mutations are produced during excision repair of alkylation damage. Increased frequencies of EMS or MMS-induced mutation in uvs9 strains could indicate that induced mutations are not produced by excision repair of alkylation damage, but might be produced by some later pathways. These later pathways could act on secondary damage as described in the model for UV-induced mutations. If so, then these pathways must be different from the rev pathways, since rev strains are not reduced in EMS-induced mutations.

V.

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VI.

REFERENCES

1. Alexander, P. and K. A. Stacey, Comparison of the changes produced by ionizing radiation and by the alkylating agents: Evidence for a similar mechanism at the molecular level, Ann. N. Y. Acad. Sci. 68, 1225-1237 (1958).

2. Averbeck, D., W. Laskowski, F. Eckardt, and E. Lehmann-Brauns, Four radiation sensitive mutants of Saccharomyces. Survival after UV- and X-ray-irradiation as well as UV-induced reversion rates from isoleucine-valine dependence to independence, Molec. Gen. Genetics 107, 117-127 (1970).

3. Baker, R. M., Stimulation of genetic recombination among lambda bacteriophages by ultraviolet radiation: The mechanism and the relevance of DNA repair processes, University of California Lawrence Radiation Laboratory Report UCRL- 19708, Ph.D. Thesis (Feb. 1970), Berkeley, California.

4. Beam, C. A., R. K. Mortimer, R. G. Wolfe, and C. A. Tobias, The relation of radioresistance to budding in Saccharomyces cerevisiae, Arch. Biochem. Biophys. 49, 110-122 (1954).

5. Bohme, H. and E. Geissler, Repair of lesions induced by photodynamic action and by ethylmethanesulfonate in E. coli, Molec. Gen. Genetics 103, 228-232 (1968).

6. Boyce, R. P. and P. Howard-Flanders, Release of ultraviolet light-induced thymine dimers from DNA in E. coli K12, Proc. Natl. Acad. Sci. 51, 293-300 (1964).

7. Bridges, B. A., Mechanisms of radiation mutagenesis in cellular and subcellular systems, Ann. Rev. Nuc. Sci. 19, 139-178 (1969).

8. Bridges, B. A., and J. J. Munson, The persistence through several replication cycles of mutation producing pyrimidine dimers in a strain of E. coli deficient in excision-repair, Biochem. Biophys. Acta. 30, 620-624 (1968).

9. Brown, D. M., and A. R. Todd, Evidence on the nature of chemical bonds in nucleic acids, p. 444, in Chargaff, E. and J. N. Davidson, The Nucleic Acids vol. 1, Academic Press, New York, pp. 409-445 (1955).

10. Chang, L. and R. W. Tuveson, Ultraviolet-sensitive mutants in Neurospora crassa, Genetics 56, 801-810 (1967).

11. Chang, L., J. E. Lennox, and R. W. Tuveson, Induced mutation in UV-sensitive mutants of Aspergillus nidulans and Neurospora crassa, Mutation Res. 5, 217-224 (1968).

12. Chen, S. Y., B. Ephrussi, and H. Hottinguer, Nature genetique des mutants a deficience respiratoire de la souche B-II de la levure de boulangerie, Heredity 4, 337-351 (1950).

13. Clark, A. J. and A. D. Margulies, Isolation and characterization of recombination-deficient mutants of Escherichia coli K12, Proc. Natl. Acad. Sci. 53, 451-459 (1965).

14. Cox, B. S. and J. M. Parry, The isolation, genetics and survival characteristics of UV light-sensitive mutants in yeast, Mutation Res. 6, 37-55 (1968).

15. Drake, J. W., Mutagenesis mechanisms, Ann. Rev. Genetics 3, 247-268 (1969).

16. Emmerson, P. T., Recombination-deficient mutants of E. coli K12 that map between thyA and argA, Genetics 60, 19-30 (1968).

17. Ephrussi, B., Nucleo-cytoplasmic Relations in Microorganisms, Clarendon Press, Oxford, England, 1953.

18. Esposito, M. S., X-ray and meiotic fine structure mapping of the adenine-8 locus in Saccharomyces cerevisiae, Genetics 58, 507-527 (1968).

18a. Fogel, S., D. D. Hurst and K. K. Mortimer, Gene conversion in unselected tetrads from multipoint crosses, Second Stadler Symposium, May 1970.

19. Freifelder, D., Mechanism of inactivation of coliphage T7 by X-rays, Proc. Natl. Acad. Sci. 54, 128-134 (1965).

20. Gilmore, R. A., Super-suppressors in Saccharomyces cerevisiae, Genetics 56, 641-658 (1967).

21. Goulian, M., and A. Kornberg, Enzymatic synthesis of DNA XXIII. Synthesis of circular replicative form of phage ØX174 DNA, Proc. Natl. Acad. Sci. 58, 1723-1730 (1967).

22. Grivell, A. R. and J. F. Jackson, Thymidine Kinase: Evidence for its absence from Neurospora crassa and some other microorganisms and the relevance of this to the specific labeling of DNA, J. Gen. Microbiol. 54, 307-317 (1968).

23. Haefner, K. and L. Howrey, Gene controlled UV-sensitivity in Schizosaccharomyces pombe, Mutation Res. 4, 219-221 (1967).

24. Harm, W., H. Harm, and C. S. Rupert, Analysis of photoenzymatic repair of UV lesions in DNA by single light flashes. II. in vivo studies with E. coli cells and bacteriophage, Mutation Res. 6, 371-385 (1968).

25. Harm, W. and B. Hillebrandt, A. non-photoreactivable mutant of E. coli B, Photochem. Photobiol. 1, 271-272 (1962).

26. Hawthorne, D. C., Identification of nonsense codons in yeast, J. Mol. Biol. 43, 71-75 (1969).

27. Hawthorne, D. C. and R. K. Mortimer, Chromosome mapping in Saccharomyces: centromere-linked genes, Genetics 45, 1085-1110 (1960).

28. Hawthorne, D. C., and R. K. Mortimer, Super-suppressors in yeast, Genetics 48, 617-620 (1963).

29. Hill, R. F., Ultraviolet induced lethality and reversion to prototrophy in Escherichia coli strains with normal and reduced dark repair ability, Photochem. Photobiol. 4, 563-568 (1965).

30. Holliday, R., Radiation-sensitive mutants of Ustilago maydis, Mutation Res. 2, 557-559 (1965).

31. Holliday, R., Altered recombination frequencies in radiation-sensitive strains of Ustilago, Mutation Res. 4, 275-288 (1967).

32. Howard-Flanders, P., Genes that control DNA repair and genetic recombination in E. coli, Adv. Biol. Med. Phys. 12, 299-317 (1968).

33. Howard-Flanders, P., DNA repair, Ann. Rev. Biochem. 37, 175-200 (1968).

34. Howard-Flanders, P., and R. P. Boyce, DNA repair and genetic recombination: studies of mutants of E. coli defective in these processes, Rad. Res. Supp. 6, 156-184 (1966).

35. Howard-Flanders, P., L. Theriot, and J. B. Stedeford, Some properties of excision-defective, recombination-defective mutants of E. coli K12, J. Bacteriol. 97, 1134-1141 (1969).

36. Jagger, J., Photoreactivation, Bacteriol. Rev. 22, 99-142, (1958).

37. Johnston, J. R., and R. K. Mortimer, Use of snail digestive juice in isolation of yeast spore tetrads, J. Bacteriol. 78, 292 (1959).

38. Jones, E., A comparative study of two adenine loci in Saccharomyces cerevisiae, Ph.D. Thesis, University of Washington, Seattle, Wash. (1964).

39. Kaplan, H. S., DNA-strand scission and loss of viability after X-irradiation of normal and sensitized bacterial cells, Proc. Natl. Acad. Sci. 55, 1442-1446 (1966).

40. Kaplan, J. C., S. R. Kushner, and L. Grossman, Enzymatic repair of DNA, 1. Purification of two enzymes involved in the excision of thymine dimers from ultraviolet-irradiated DNA, Proc. Natl. Acad. Sci. 63, 144-151 (1969).

41. Kelly, R. B., M. R. Atkinson, J. A. Huberman, and A. Kornberg, Excision of thymine dimers and other mismatched sequences by DNA polymerase of E. coli, Nature 224, 495-501 (1969).

42. Kelner, A., Effect of visible light on the recovery of Streptomyces griseus conidia from ultraviolet irradiation injury, Proc. Natl. Acad. Sci. 35, 73-79 (1949).

43. Kelner, A., Photoreactivation of ultraviolet-irradiated Escherichia coli with special reference to the dose-reduction principle and to ultraviolet-induced mutation, J. Bacteriol. 58, 511-522 (1949).

44. Kondo, S., and J. Jagger, Action spectra for photoreactivation of mutation to prototrophy in strains of E. coli possessing and lacking photoreactivating enzyme activity, Photochem. Photobiol. 5, 189-200 (1966).

45. Kondo, S., and T. Kato, Action spectra for photoreactivation of killing and mutation to prototrophy in UV-sensitive strains of E. coli possessing and lacking photoreactivating enzyme, Photochem. Photobiol. 5, 827-837 (1966).

46. Kondo, S., and T. Kato, Photoreactivation of mutation and killing in Escherichia coli, Adv. Biol. Med. Phys. 12, 283-298 (1968).

47. Lanier, W. B., R. W. Tuveson and J. E. Lennox, A radiation-sensitive mutant of Aspergillus nidulans, Mutation Res. 5, 23-31 (1968).

48. Lemontt, J. F., this study.

49. Lemontt, J. F., and R. K. Mortimer, The selection of mutants of yeast that are defective in ultraviolet light-induced mutation (abstract), Genetics 64, s37 (1970).

50. Lennox, J. E., and R. W. Tuveson, The isolation of ultraviolet sensitive mutants from Aspergillus rugulosus, Radiation Res. 31, 383-388 (1967).

51. Lindegren, G., Y. L. Hwang, Y. Oshima, and C. C. Lindegren, Genetical mutants induced by ethylmethanesulfonate in Saccharomyces, Canad. J. Genet. Cytol. 7, 491-499 (1965).

52. Loprieno, N., S. Bonatti, A. Abbondandolo, and R. Guglielminetti, The nature of spontaneous mutations during vegetative growth in Schizosaccharomyces pombe, Molec. Gen. Genetics 104, 40-50 (1969).

53. Magni, G. E., and P. P. Puglisi, Mutagenesis of super-suppressors in yeast, Cold Spring Harbor Symp. Quant. Biol. 31, 699-704 (1966).

53a. Magni, G. E., R. C. von Borstel, and C. M. Steinberg, Super-suppressors as addition-deletion mutations, J. Mol. Biol. 16, 568-570 (1966).

54. Manney, T. R., Action of a super-suppressor in yeast in relation to allelic mapping and complementation, Genetics 50, 699-704 (1966).

55. Manney, T. R., and R. K. Mortimer, Allelic mapping in yeast by X-ray-induced mitotic reversion, Science 143, 581-582 (1964).

56. McClary, D. O., W. L. Nulty, and G. R. Miller, Effect of potassium versus sodium in the sporulation of Saccharomyces, J. Bacteriol. 78, 362-368 (1959).

57. McGrath, R. A., and R. W. Williams, Reconstruction in vivo of irradiated E. coli deoxyribonucleic acid; the rejoining of broken pieces, Nature 212, 534-535 (1966).

58. Miura, A., and J. Tomizawa, Studies on radiation-sensitive mutants of E. coli.III. Participation of the Rec system in induction of mutation by ultra-violet irradiation, Molec. Gen. Genetics 103, 1-10 (1968).

59. Mortimer, R. K., unpublished data.

60. Mortimer, R. K., and R. A. Gilmore, Suppressors and suppressible mutations in yeast, Adv. Biol. Med. Phys. 12, 319-331 (1968).

61. Mortimer, R. K., and D. C. Hawthorne, Genetic mapping in Saccharomyces, Genetics 53, 165-173 (1966).

62. Mortimer, R. K., F. Sherman, and R. C. von Borstel, Yeast genetics supplement on nomenclature, Microbial Genetics Bulletin, no. 31 (Nov. 1969).

63. Mounolou, J. C., H. Jakob, and P. P. Slonimiski, Mitochrondrial DNA from yeast "petite" mutants: Specific changes of buoyant densities corresponding to different cytoplasmic mutations, Biochem. Biophys. Res. Commun. 24, 218-224 (1966).

64. Moustacchi, E., Cytoplasmic and nuclear genetic events induced by UV light in strains of Saccharomyces cerevisiae with different UV-sensitivities, Mutation Res. 7, 171-185 (1969).

65. Muller, H. F., Artificial transmutation of the gene, Science 66, 84-87 (1927).

66. Nakai, S., and S. Matsumoto, Two types of radiation-sensitive mutants in yeast, Mutation Res. 4, 129-136 (1967).

67. Nakai, S., and R. K. Mortimer, Studies of the genetic mechanism of radiation-induced mitotic segregation in yeast, Molec. Gen. Genetics 103, 329-338 (1969).

68. Nasim, A., Repair mechanisms and radiation-induced mutations in fission yeast, Genetics 59, 327-333 (1968).

69. Olivera, B. M., and I. R. Lehman, Linkage of polynucleotides through phosphodiester bonds by an enzyme from Escherichia coli, Proc. Natl. Acad. Sci. 57, 1426-1433 (1967).

70. Patrick, M. H., and R. H. Haynes, Dark recovery phenomena in yeast. II. Conditions that modify the recovery process, Radiation Res. 23, 564-579 (1964).

71. Patrick, M. H., R. H. Haynes, and R. B. Uretz, Dark recovery phenomena in yeast. I. Comparative effects with various inactivating agents, Radiation Res. 21, 144-163 (1964).

72. Perkins, D. D., Biochemical mutants in the smut fungus Ustilago maydis, Genetics 34, 607-626 (1949).

73. Pettijohn, D., and P. Hanawalt, Evidence for repair-replication of UV-damaged DNA in bacteria, J. Mol. Biol. 9, 395-410 (1964).

74. Raut, D., Heritable non-genic changes induced in yeast by ultraviolet light, J. Cellular Comp. Physiol. 44, 463-475 (1954).

75. Reaume, S. E., and E. L. Tatum, Spontaneous and nitrogen mustard-induced nutritional deficiencies in Saccharomyces cerevisiae., Archs. Biochem. 22, 331-338 (1949).

76. Resnick, M. A., Genetic control of lethality and mutation in Saccharomyces cerevisiae, University of California Lawrence Radiation Laboratory Report UCLRL-18404, Ph.D. Thesis (Aug. 1968), Berkeley, California.

77. Resnick, M. A., A photoreactivationless mutant of Saccharomyces cerevisiae, Photochem. Photobiol. 9, 307-312 (1969).

78. Resnick, M. A., Genetic control of radiation sensitivity in Saccharomyces cerevisiae, Genetics 62, 519-531 (1969).

79. Resnick, M. A., Induction of mutations in Saccharomyces cerevisiae by ultraviolet light, Mutation Res. 7, 315-332 (1969).

80. Roman, H., A system selective for mutations affecting the synthesis of adenine in yeast, Compt. Rend. Trav. Lab., Carlsberg 26, 299-314 (1956).

81. Rorsch, A., P. van de Putte, I. E. Mattern, and H. Zwenk, Genetic and enzymic control of radiation sensitivity in E. coli, In Proceedings of the Conference on Genetical Aspects of Radiation Sensitivity: Mechanisms of Repair, pp. 105-129, International Atomic Energy Agency, Vienna (1966).

82. Rupert, C. S., and W. Harm, Reactivation after photobiological damage, In Advances in Radiation Biology, vol. 2, pp. 1-81, Academic Press, New York (1966).

83. Rupp, W. D., and P. Howard-Flanders, Discontinuities in the DNA synthesized in an excision-defective strain of E. coli following ultraviolet irradiation, J. Mol. Biol. 31, 291-304 (1968).

84. Setlow, J. K., The molecular basis of biological effects of ultraviolet radiation and photoreactivation, In Current Topics in Radiation Research, vol. 2, pp. 197-248 (1966).

85. Setlow, R. B., Cyclobutane-type pyrimidine dimers in polynucleotides, Science 153, 379-386 (1966).

86. Setlow, R. B., The photochemistry, photobiology and repair of polynucleotides, In Prog. in Nucleic Acid Research and Molecular Biol., vol. 8, pp. 257-295, Academic Press, New York (1968).

87. Setlow, R. B., and W. L. Carrier, The disappearance of thymine dimers from DNA: An error correcting mechanism, Proc. Natl. Acad. Sci. 51, 226-231 (1964).

88. Sherman, F., personal communication.

89. Sherman, F., and P. P. Slonimski, Respiration-deficient mutants of yeast. II. Biochemistry, Biochem. Biophys. Acta 90, 1-15 (1964).

90. Smirnov, M. N., V. N. Smirnov, E. I. Budowsky, S. G. Inge-Vechtomov, and N. G. Serebrjakov, Red pigment of adenine-deficient yeast Saccharomyces cerevisiae, Biochem. Biophys. Res. Commun. 27, 299-304 (1967).

91. Smith, K. C., Biologically important damage to DNA by photoproducts other than cyclobutane-type thymine dimers, In Radiation Research, pp. 756-770, North Holland Publishing Co., Amsterdam (1967).

92. Smith, K. C., Dark repair of DNA damage, presented at the NATO Advanced Study Institute of Biological Effects of Visible Light, Sept. 3-17, Alghero, Sardinia, Italy (1969).

93. Smith, K. C., and D. H. C. Meun, Repair of radiation-induced damage in Escherichia coli. I. Effect of rec mutations on post-replication repair of damage due to ultraviolet irradiation, J. Mol. Biol., in press.

94. Snow, R., Mutants of yeast sensitive to ultraviolet light, J. Bacteriol. 94, 571-575 (1967).

95. Snow, R., Recombination in UV-sensitive strains of Saccharomyces cerevisiae, Mutation Res. 6, 409-418 (1968).

96. Srb, A. M., R. D. Owen and R. S. Edgar, General Genetics, second edition, W. H. Freeman, San Francisco (1965).

97. Stadler, L. J., Mutations in barley induced by X-rays and radium, Science 68, 186-187 (1928).

98. Strauss, B. S., Differential destruction of the transforming activity of damaged deoxyribonucleic acid by a bacterial enzyme, Proc. Natl. Acad. Sci. 48, 1670-1675 (1962).

99. Strauss, B. S., DNA repair mechanisms and their relation to mutation and recombination, In Current Topics in Microbiology and Immunology, Springer-Verlag, New York (1968).

100. Van de Putte, P., and A. Rorsch, Radiation-sensitive mutants of E. coli defective in genetic recombination, In Regulation of Nucleic Acid and Protein Biosynthesis, Koningsberger and Bosch (eds.), pp. 63-67, vol. 10, Elsevier Publishing Co., Amsterdam (1967).

101. Wacker, A., Molecular mechanisms of radiation effects. In Prog. Nucleic Acid Research and Molecular Biol., vol. 1, pp. 369-399, Academic Press, New York (1963).

102. Wildenberg, J., Gene conversion in synchronous yeast cell pedigrees, Ph.D. thesis (1969), City University of New York.

103. Wilkie, D., The induction by monochromatic u.v. light of respiratory-deficient mutants in aerobic and anaerobic cultures of yeast, J. Mol. Biol. 7, 527-533 (1963).

104. Witkin, E. M., Photoreversal and "dark repair" of mutations to prototrophy induced by ultraviolet light in photoreactivable and non-photoreactivable strains of Escherichia coli, Mutation Res. 1, 22-36 (1964).

105. Witkin, E. M., Mutation and the repair of radiation damage in bacteria, Radiation Res. Suppl. 6, 30-53 (1966).

106. Witkin, E. M., Mutation-proof and mutation-prone modes of survival in derivatives of E. coli B differing in sensitivity to ultraviolet light, Brookhaven Symposia in Biology 20, 17-55 (1967).

107. Witkin, E. M., Ultraviolet-induced mutation and DNA repair, Ann. Rev. Genet. 3, 525-552 (1969).

108. Witkin, E. M., The mutability toward ultraviolet light of recombination-deficient strains of E. coli, Mutation Res. 8, 9-14 (1969).

109. Witkin, E. M., N. A. Sicurella, and G. M. Bennett, Photoreversibility of induced mutations in a non-photoreactivable strain of Escherichia coli, Proc. Natl. Acad. Sci. 50, 1055-1059 (1963).

110. Wohlrab, G., and R. W. Tuveson, Effects of liquid holding on the induction of mutations in an ultraviolet light-sensitive strain of Aspergillus nidulans, Mutation Res. 8, 265-275 (1969).

111. Zakharov, I. A., T. N. Kozina, and I. V. Federova, Effets des mutations vers la sensibilite au rayonnement ultraviolet chez la levure, Mutation Res. 9, 31-39 (1970).

112. Zimmermann, F. K., Sensitivity to methylmethanesulfonate and nitrous acid of ultraviolet light-sensitive mutants in Saccharomyces cerevisiae, Molec. Gen. Genetics 102, 247-256 (1968).

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